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ANNALS OF BOTANY

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On the Water Relations of Plant Cells

BY

D. THODAY

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With one Figure in the Text

IN a recent paper Burström (1948) has maintained that, when a vacuolated cell is absorbing water, the turgor pressure must be greater than the wall pressure since the wall is being further distended. Surely there is a confusion of thought here. Since action and reaction are equal and opposite, the pressure of the wall and the hydrostatic pressure of the contents must be equal at all stages in the process. Whatever brings about the increase in wall pressure causes also the increase in hydrostatic pressure. The case is analogous to that of a pneumatic tyre being pumped up: the air pressure and the opposing pressure of the distended tyre are equal and opposite at every stage and both are increased together by the action of the pump.

Similarly, in Pfeffer's osmotic apparatus, before the pressure in the manometer reaches the maximum value which, by definition, measures the osmotic pressure of the solution, the manometer measures at any intermediate stage the hydrostatic pressure already reached in the osmotic cell at that stage: this pressure is equal and opposite to the manometric pressure and to the back pressure of the wall of the vessel and its membrane on the solution. The lack of equilibrium which renders the system a dynamic one is due to the osmotic *potentialities* of the solution.

To resolve the confusion all that seems necessary is to recognize that osmotic pressures and suction pressures (likewise imbibition pressures) are potentials, which are only made evident by actual pressures under appropriate conditions (cf. Beck, 1928, p. 415). In a cell at the stage of incipient plasmolysis, or in a cell in air that has lost water by evaporation till the wall is just completely relaxed, neither wall pressure nor turgor pressure exists, but the osmotic potential of the cell is available as a suction potential if the cell is put into effective communication with a source of water at a lower suction potential than its own. The difference of suction potential plays a part analogous to that of the tyre-pump. The maximum pressure that the tyre-pump will provide corresponds to the osmotic pressure of the solution, which expresses the limit of effectiveness of its osmotic potential.

When a cell has reached equilibrium with a solution in which it is immersed, the osmotic potential of the cell-sap is in part balanced by the osmotic potential of the external solution, in part expressed in the turgor pressure which is balanced by the wall pressure. If the cell is removed from the

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solution, the former part is available as a suction potential which becomes effective when the cell is immersed in water.

These relations hold whatever the nature of the potential differences that come into play. If, as appears to be indicated by Bennet-Clark's experiments, cells can hold water against an external solution of higher osmotic pressure than that of their sap, there must exist in addition to ordinary osmotic potentials other potentials, such as electrosmotic potentials maintained by the activity of the protoplasm. The suction potential of the cell would then be of composite origin; but the equilibrium relations could still be expressed by the usual equations, for example,

$$P-T = p = S,$$

where P represents the potential of the cell (in this case partly osmotic, partly electrosmotic), p that of the external solution, T the turgor pressure, and S the suction potential of the cell. Alternatively a separate expression could be added, say, E, for the additional factor, leaving P for the ordinary osmotic potential, so that $(P+E)-T = p = S$.

When, however, we attempt to envisage the physical meaning of the balanced potentials at the molecular level, we are on less sure ground. The osmotic potential is related to the concentration of the solute molecules and ions but independent of their natures. Electrosmotic potentials depend on the nature of the solvent and of the membrane permeable to it, which determine the electrical charge on the solvent molecules in the membrane; also on the processes that maintain the difference of electrical potential between the two sides of the membrane. The situation is evidently complex. It is not, however, necessary to adopt particular views of these mechanisms in order to express the hydrostatic conditions that result from their action.

Meyer (1938) prefers the concept of water 'diffusion pressure' to osmotic pressure or suction pressure as having particular reference to the solvent. Taking the diffusion pressure of pure water as the standard of reference, he substitutes 'diffusion pressure deficit' for osmotic pressure. This term is also liable to be misleading unless it is recognized that this also signifies a potential which, like osmotic potentials and suction potentials, is measured in terms of hydrostatic pressures, after establishing equilibrium conditions in which the potential difference is nil. Meyer, in fact, defines 'diffusion pressure deficit' as equalling the external pressure required to raise the diffusion pressure of the solution to that of the pure solvent.

'Diffusion pressure' has the merit that it is related directly to the pressure of water vapour which would be in equilibrium with the solution or cell. This relation is made use of in Barger's well-known method for determining osmotic pressures of small quantities of fluid, on the principle that solutions in equilibrium with the same pressure of water vapour would be in osmotic equilibrium with one another if brought into communication through a semipermeable membrane. Vapour pressure being taken as an index, the water 'diffusion pressure' of a solution will be increased by hydrostatic pressure:

thus in a fully turgid cell the turgor pressure increases the water diffusion pressure from that of the uncompressed sap to that of pure water. How this change is to be envisaged at the molecular level is again another question.

Burström states (p. 61) that 'In the cell we have only two actual pressures, the turgor pressure, equalling the difference in D.P. (diffusion pressure) of the water on the two sides of the membrane, and the wall pressure, caused by the tension in the membrane. These two pressures are of fundamentally different physical origin, and each may attain any value independent of the other.' Here he confuses the issue by departing from the customary use of the term turgor pressure and applying it to the difference of diffusion potential which is not a pressure in the same sense. It is able to develop a pressure and do work under appropriate conditions; but the only two actual pressures in the strict, hydrostatic sense are the wall pressure and the turgor pressure, which are equal and opposite. Burström also states (p. 60) that when a sugar solution is separated from water by a movable semipermeable membrane, the pressure difference causing the membrane to move can be computed and equals the diffusion pressure deficit. There is a fallacy here. The pressure moving the membrane is *caused* by the 'diffusion pressure deficit' or difference of osmotic potential, but the two are not identical. The effective pressure will depend on the frictional resistance offered to the movement of the membrane. To measure the 'diffusion pressure deficit' it would be necessary to measure the resistance offered by the membrane to the movement of water and also the rate of flow. The case is analogous to that of an electrical system in which the difference of electric potential can be determined if the current and the resistance can be measured, from the relation $C = \frac{E}{R}$.

Similarly, when the potential difference is known and the rate of flow can be measured, the resistance to flow can be obtained. This is the principle of the plasmolytic method for determining the permeability of protoplasm, which is inversely proportional to the resistance.

The point of view here stated may be conveniently summarized by reference to the three methods available in theory for determining the condition of a plant cell in relation to the retention or absorption of water:

1. The pressure of water vapour in equilibrium with it may be determined. The suction potential of the cell is equal to the osmotic potential of solutions with the same vapour tension.

2. The cell may be compressed and the pressure noted at which water just begins to exude: if in this condition the cell were brought into contact with pure water it would neither take up water nor yield water.

The equilibrium would be represented by the equation:

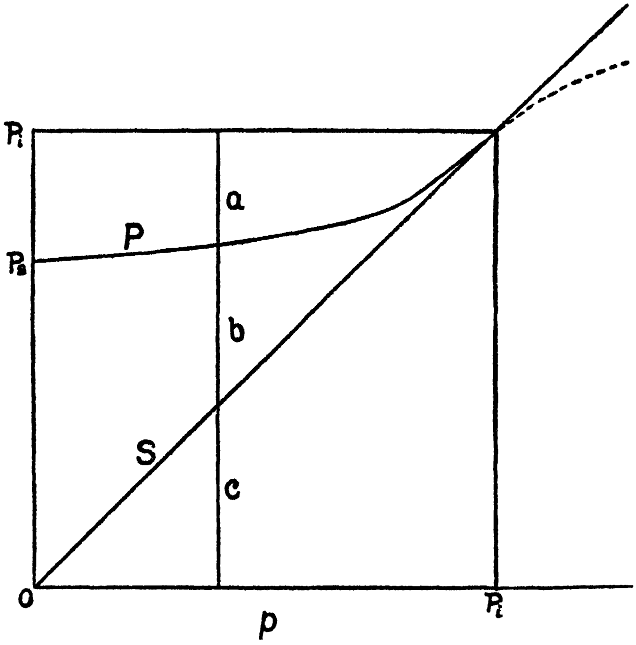
$$P - W - e = 0,$$

where P = the osmotic potential of the cell-sap, W = the initial wall pressure, e = the pressure applied externally.

The hydrostatic pressure within the cell would be $W+e$.[1]

The water diffusion pressure of the system would be equal to that of pure water, the diffusion pressure deficit nil.

Further compression would lead to exudation of water and an increase in concentration of the sap, P increasing therefore to balance the increase in e.



The abscissae represent the osmotic potential of the external solution, p, rising from zero to P_i, the osmotic potential of the cell-sap at incipient plasmolysis. Ordinates represent cell potentials. S, the suction potential, is equal to p. P varies from P_i at incipient plasmolysis (on the right) to a value at full turgor depending on the degree of dilution, i.e. on the expansion of the volume of the vacuole: the saturation value $P_s = P_i \times \frac{V_i}{V_s}$.

The form of the P-curve between these two points will depend on the exact course of volume-change. Tamiya points out that whereas Ursprung and Blum assumed proportionality between increase of volume and turgor pressure, measurements by various experimenters show greater extensibility at low turgor pressures and increasing resistance to further distension as turgor increases. The degree of curvature is therefore greater to the right, and diminishes to the left as full turgor is approached.[2]

At any intermediate point between $p = 0$ and $p = P_i$, the ordinate is divided by the P- and S-curves into three portions. Of these, $c = S$, $b = P-S =$ the turgor pressure, a is the loss of osmotic potential of the sap resulting from dilution.

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[1] Actually this is a simplification. Compression would increase the hydrostatic pressure, which would involve an increase in the wall pressure in directions other than the direction of compression. Tissue tensions involve similar relations. In a tissue confined within a resistant layer, the latter exerts a pressure on the confined tissue, but the cell-walls where they abut on air spaces bulge into the spaces and there exert a back pressure on the cell contents equal and opposite to the full hydrostatic pressure within the cell.

[2] The P-curve in the figure has been recalculated from data obtained for *Nitella* by Stow quoted by Tamiya (1938).

3. A solution of a suitable solute (usually sucrose) may be found of such a concentration that no water passes either way when the cell is immersed in it. In this case the equilibrium would be represented by the equation:

$$P-W=p,$$

where p = the osmotic potential of the external solution.

Here the hydrostatic pressure in the cell is the original turgor pressure $T=W$.

The diffusion pressure deficit is equal to p.

In both 2 and 3, at equilibrium, the 'net suction pressure' of the system (Stiles, 1924, p. 98), i.e. the potential difference, is nil; but whereas the suction potential of the cell in 2 is zero, in 3 it is equal to p.

If a difference of potential exists between the cell and its environment it will cause movement of water into or out of the cell. Though the conditions are changing, the condition of the cell at any given moment can nevertheless be expressed in terms of a similar hypothetical equilibrium. The suction potential of the cell, S, will be $P-W$; the difference of potential, or net suction pressure, will be $S-p$.

The extent of the changes occurring in the attainment of equilibrium depends on the extensibility of the cell wall. This aspect has been specially considered by Sakamura (1937) and Tamiya (1938) and expressed in a novel form of diagram, which is reproduced here from Tamiya with modified lettering.

As pointed out by these and other authors, the validity of the relations expressed depends on the temperature and the value of P_i remaining constant.

For cells actually immersed in solutions the diagram can be extended to the right, the S-curve for the plasmolysed cell continuing in a straight line, with P coincident with it.

If the diagram is used to represent the water relations of cells not immersed but in contact with air, the value of p can be determined from the equilibrium vapour tension. Here again the diagram can be extended to the right, but the extensions of the P- and S-curves will not necessarily coincide. If the wall resists collapse, S will increase faster than P. S being related to the vapour tension and so to p, the S-curve will continue straight as the reference line; the P-curve will cross it (cf. Meyer, 1938, p. 544).

SUMMARY

It is suggested that recurrent confusion and misunderstanding could be avoided by treating osmotic and suction pressures as potentials, in contrast with hydrostatic pressures set up by them.

Attention is drawn to a diagrammatic representation of the relations between these various quantities in systems including plant cells, adopted with modification by Tamiya from Sakamura.

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Plant Populations

II. The Estimation of the Number of Individuals per Unit Area of Species in Heterogeneous Plant Populations

BY

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With three Figures in the Text

I. INTRODUCTION

IT has been shown that if the individuals of a plant species are distributed at random an estimation of the density or number of individuals per quadrat may be obtained by observing only the presence or absence of the species in the quadrat (Blackman, 1935). If m is the mean number of individuals per quadrat, then the first term of the Poisson series gives the number of quadrats which do not contain the species and hence

$$m = -\log_e \frac{n_0}{N},$$

where n_0 is the number of quadrats with no individuals and N is the total number of quadrats.

However, if the population is a heterogeneous one in which the variance is significantly greater than the mean, then two or more parameters are needed to describe the distribution and consequently an estimate of the mean density can no longer be obtained from the simple equation of the first term of the series. This difficulty is encountered when the observed distribution can be fitted by Neyman's Contagious series (Neyman, 1939; Archibald, 1948). The basic hypothesis of this series is that the individuals occur in clusters which are distributed at random, the number of individuals per cluster also being a random number—an hypothesis which is in harmony with the known facts of plant propagation. However, the premisses on which Neyman's series was based were necessarily more complicated than those pertaining to plant populations, and Thomas (1949) has now evolved another form of the double Poisson series which, while it retains the original idea of contagion, expresses the ultimate series in a simpler form. It will be shown that by observing the number of quadrats with 0 and 1 individuals the parameters of this new series can be obtained approximately, and that they give a good estimation of the number of individuals per quadrat for heterogeneous populations.

Before considering the actual estimation of density a brief statement of the new series is given with an example to show how it may be fitted.

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II. THOMAS'S DOUBLE POISSON SERIES

In evolving a series for describing the distribution of plants when sampled by the quadrat method Thomas assumes that the individuals of a species are distributed in a heterogeneous manner, showing a certain degree of aggregation or clustering, and that (i) the number of clusters per quadrat are distributed at random with a mean m, and (ii) the number of units per cluster, additional to the first unit, is also a random number with parameter λ, and hence the mean number of units per cluster is $(1+\lambda)$.

The terms of the series are given by

$$P\{0\} = e^{-m}, \tag{1}$$

$$P\{k\} = \sum_{r=1}^{k} \frac{e^{-m}m^r}{r!}\left\{e^{-r\lambda}\frac{\lambda^{(k-r)}r^{(k-r)}}{(k-r)!}\right\}, \tag{2}$$

where $k = 1, 2, 3,\ldots$ gives the successive terms of the series and $\sum\limits_{r=1}^{k}$ stands for the summation of all values from $r = 1$ to $r = k$.

The values of m and λ are obtained from the first and second moments as follows:

$$\mu_1 = m(1+\lambda), \tag{3}$$

$$\mu_2 = m(1+3\lambda+\lambda^2), \tag{4}$$

where μ_1 and μ_2 are respectively equal to the mean and variance of the sample population.

A method of fitting the series to field data

The series may be conveniently described as a Double Poisson series since it is the sum of the product of the terms of two simple Poisson series $\frac{e^{-m}m^r}{r!}$ and $e^{-r\lambda}\frac{\lambda^{(k-r)}r^{(k-r)}}{(k-r)!}$. The terms of these two simple Poisson series for different values of k and r may be obtained by interpolation from Table LI for the Poisson series in 'Tables for Statisticians and Biometricians' (1945).

This method of fitting the series is shown in Table I, where data for *Carex flacca* is used. The observed and calculated frequencies are then compared by means of the χ^2 test, and the probability value, $P(\chi^2) = 0{\cdot}44$, indicates that they do not differ significantly. It has also been shown by Thomas that the series gives a good fit for data for *Armeria maritima* and *Plantago maritima* supplied by the writer.

III. THE ESTIMATION OF DENSITY FROM FIELD OBSERVATIONS

To obtain an estimate of density from this new series it is necessary to know only the number of quadrats with no individuals and the number with one individual. Then from the first two terms of the series we have

$$P\{0\} = e^{-m} \tag{5}$$

and

$$P\{1\} = me^{-(m+\lambda)}, \tag{6}$$

and the two parameters can be estimated from

$$m = -\log_e \frac{n_0}{N} \tag{7}$$

and

$$\lambda = -\log_e \frac{n_1}{mn_0}, \tag{8}$$

where n_0 is the number of quadrats with no individuals, n_1 is the number of quadrats with only one individual, and N is the total number of quadrats.

Then $m(1+\lambda)$ is the estimated mean number of individuals per quadrat.

TABLE I

Method for fitting Thomas's Series. Data for Carex flacca
(Chalk grassland, Pitstone, May 1947)

Number of quadrats, 500; quadrat size, 20 sq. cm. $m = 1·055$; $\lambda = 0·3383$ (0·34 approx.)

		$e^{-r\lambda}\lambda^{(k-r)}r^{(k-r)}/(k-r)!$							
r	$\frac{e^{-m}m^r}{r!}$	k 0	1	2	3	4	5	6	λ
0	0·348625								
1	0·366933	0·712508	0·240598	0·041452	0·004859	0·000434	0·000031	0·000002	1
2	0·193535		0·496030	0·343945	0·116087	0·026662	0·004567	0·000628	2
3	0·068203			0·364378	0·367707	0·185685	0·062566	0·015826	3
4	0·018066				0·259565	0·349764	0·235977	0·106284	4
5	0·003837					0·184605	0·311809	0·263423	5
6	0·000680						0·131471	0·266617	6
7	0·000104							0·094530	7
	Product:	0·26143	0·088283	0·015209	0·001783	0·000159	0·000010	0·000007	
			0·095997	0·066563	0·022467	0·005159	0·000884	0·000121	
				0·024851	0·025078	0·012664	0·004267	0·001078	
					0·004689	0·006318	0·000426	0·001918	
						0·000708	0·001196	0·001010	
							0·000089	0·000181	
								0·000010	
	Sum.	0·26143	0·184280	0·106623	0·054017	0·025008	0·006872	0·004325	

Terms of series:

Frequency class .	0	1	2	3	4	5	6	7	8 and over
Observed . .	181	118	97	54	32	9	5	3	1
Calculated . .	174·31	130·72	92·14	53·31	27·01	12·50	3·45	2·16	4·4

$\chi^2 = 3·76$. Degrees of freedom: $7-3 = 4$. $P(\chi^2) = 0·44$.

In Table II and Fig. 1 the observed and calculated results for sixteen species from five different communities are compared, the observation having been made with quadrats of 20 sq. cm. taken contiguously (Archibald, 1948). The coefficient of dispersion or relative variance (ratio of variance to mean) for each species is also given. If we assume with Blackman (1942) that the distribution is not a random one when the coefficient of dispersion is greater or less than unity by $2\sqrt{\left\{\frac{2N}{(N-1)^2}\right\}}$ it will be seen that the parameters of Thomas's series when derived from the first two frequency classes of the distribution give an excellent estimate of density except in the case of *Plantago media*, *P. lanceolata*, and *Helictotrichon pubescens* where the difference between n_0 and n_1 is large. It is possible that for these three species a larger quadrat would give a better estimate of density since the ratio between n_0 and n_1 would then be reduced.

When the coefficient of dispersion is not significantly different from unity, as in the case of *Glaux maritima* and *Thymus serpyllum*, the probability of a random distribution is indicated. This is confirmed by the values for the parameters, for we get a negative value for λ, and $m(1+\lambda)$ is less than unity, indicating that the double Poisson should be discarded and that a simple Poisson, value for m, would give a good estimate for density.

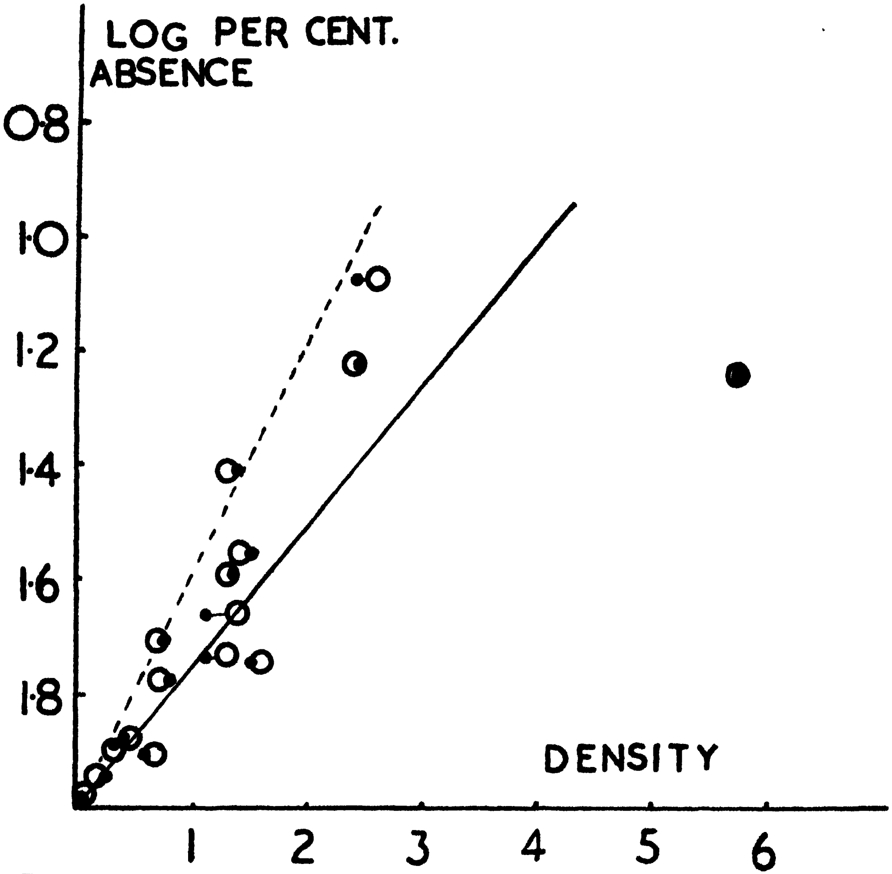

FIG. 1. The relationship between the number of individuals per quadrat (density) and the logarithm of percentage absence for different species (cf. Table II).

○ Observed density; ● density estimated according to Thomas's series. —— Regression line for estimated density according to Thomas's series coincident with regression line for field data. ---- Regression line for estimated density according to a simple Poisson series.

A comparison of the observed and estimated densities may be made if we assume with Blackman (1935, 1942) that the slope of the regression line of log percentage absence on density is given by the ratio

$$\frac{\text{mean log percentage absence}}{\text{mean density}}.$$

TABLE II

Estimation of Density (Quadrat size, 20 sq. cm.)

Species.	Total no. of quadrats.	Frequency class.		Thomas's parameters.		Density.		Coefficient of dispersion.*
		0.	1.	*m*.	(1 + λ).	Observed.	Calculated.	
Glaux maritima	500	1	15	6·214	0·119	5·796	0·738	1·107
Glaux maritima	100	26	36	1·347	0·972	1·260	1·310	0·883
Plantago coronopus	500	274	71	0·601	1·842	1·310	1·107	3·231
Carex arenaria	500	86	105	1·760	1·365	2·426	2·402	1·651
Aster tripolium	100	25	45	0·796	1·362	1·360	1·088	4·870
Plantago media	500	485	13	0·030	1·127	0·042	0·034	1·642
Plantago lanceolata	500	449	41	0·153	1·517	0·130	0·232	1·516
Helictotrichon pubescens	500	457	34	0·089	1·188	0·011	0·106	9·886
Helictotrichon pratense	500	354	58	0·345	1·745	0·658	0·602	1·941
Ranunculus bulbosus	500	368	94	0·306	1·183	0·372	0·362	0·509
Briza media	500	237	110	0·751	1·481	1·182	1·113	2·281
Carex flacca	500	181	118	1·016	1·444	1·412	1·467	1·590
Hieracium sp.	100	77	17	0·261	1·169	0·310	0·305	1·335
Briza media	100	65	18	0·431	1·212	0·610	0·522	1·603
Carex sp.	100	53	32	0·634	1·065	0·700	0·676	1·271
Thymus serpyllum	100	52	32	0·654	1·061	0·670	0·694	0·987
Festuca sp.	100	18	3	1·714	3·340	5·710	5·725	4·056
Bromus erectus	100	12	22	2·120	1·136	2·610	2·410	1·360

* Coefficient of dispersion is significantly different from unity for 500 quadrats when > or < 1 ± 0·126 and for 100 quadrats when > or < 1 ± 0·281.

Using this method the results for the observed and calculated densities in Tables II and V are compared in Figs. 1 and 2. The values from which the regression lines are obtained are given in Table III.

TABLE III

Regression of Log Percentage Absence on Density

Information.	No. of species.	Quadrat size, sq. cm.	Mean log % absence.	Mean density.		Mean parameters Thomas's series.	
				Observed.	Calculated.	*m*.	(1 + λ).
Table II	17	20	1·669	1·222	1·184	0·765	1·424
Table V	12	20	1·640	1·610	1·679	0·828	1·600
Table V	12	25	1·491	3·226	2·569	1·117	1·904

It will be seen that Thomas's series gives a good estimate for the density of the species in Table II whereas the simple Poisson series, for which the mean is given by the parameter *m* in Thomas's series, shows a considerable error. For the species in Table V there is a close agreement between the observed and estimated densities when a quadrat of 20 sq. cm. is used, but it is not so good for a quadrat of 25 sq. cm. These results are analysed more fully in a subsequent section when the values of the parameters *m* and $(1+\lambda)$ for different quadrat sizes are compared. It is, however, to be expected that the slope of the regression line should be affected by the quadrat size and that an increase in quadrat size will be accompanied by an increase in error of the estimation of density. In other words, the agreement between the observed and calculated results can only be expected for a limited arc of the regression line.

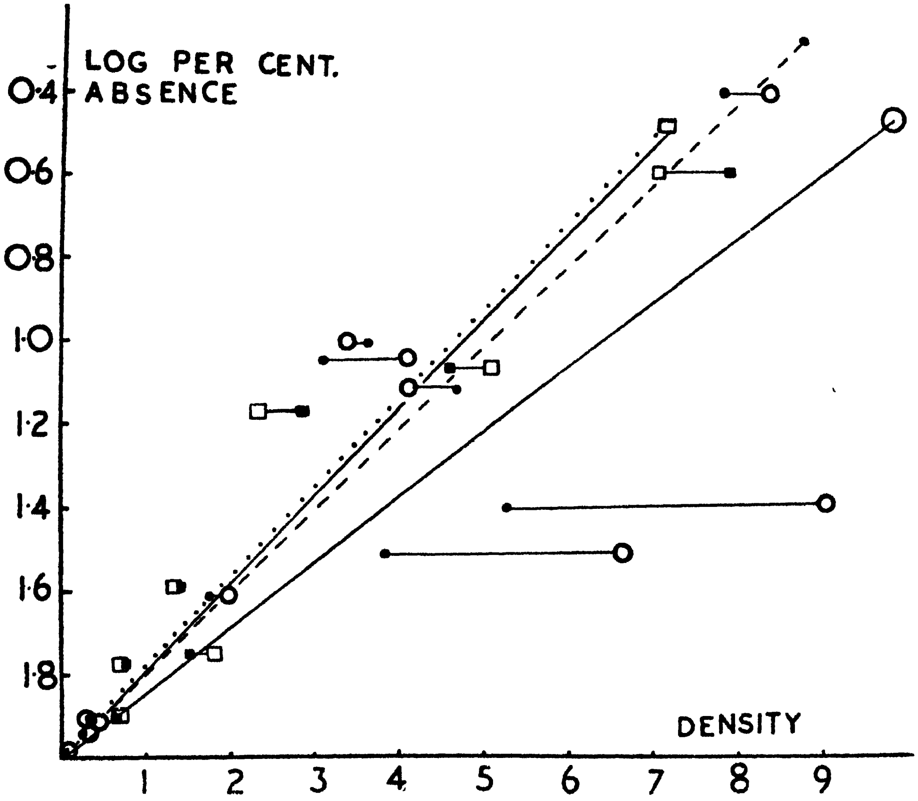

Fig. 2. Comparison of observed and estimated values for the number of individuals per quadrat of different species for two sizes of quadrat. Results indicate that a better estimate is obtained with the smaller quadrat (cf. Table V).

20 sq. cm. quadrats	*25 sq. cm. quadrats*
□ Observed density.	○ Observed density.
□— Regression line.	○— Regression line.
■ Estimated density (Thomas's series).	● Estimated density (Thomas's series).
... Regression line.	●-- Regression line.

Standard errors of estimated values

When the parameters m and λ are obtained from the first and second moments of the distribution the mean, $m(1+\lambda)$, is equal to the sample mean since the first and second moments are respectively equal to the mean and variance of the sample distribution. When, however, the parameters are estimated from the first and second frequency classes of the distribution they will be subject to error and the estimated density will also be subject to error. This is illustrated in Table IV, where the results given by the two methods are compared.

TABLE IV

Species.	Parameters from μ_1 and μ_2. *m.*	$(1+\lambda)$.	Density.	Parameters estimated from 0's and 1's.		Estimated density.	Observed density.
Briza media	0·6465	1·8284	1·182	0·751	1·484	1·127	1·182
Carex flacca	1·0551	1·3383	1·412	1·016	1·444	1·467	1·412
Armeria maritima	0·5735	2·7549	1·580	0·562	2·675	1·504	1·580
Triglochin maritimum	1·0300	1·2808	1·320	0·941	1·468	1·382	1·320

The method for determining the standard errors of the estimated parameters and the estimated density when derived from the first and second frequency classes of the distribution is given by Thomas (1949). She also gives tables for these quantities which are reproduced for the convenience of botanists in the Appendix of the present paper, their use being illustrated by reference to examples from plant populations.

The error in the estimation of density is partly dependent on the ratio n_0/n_1 which is in turn affected by quadrat size. From the practical point of view it is useful to have some criterion for the most suitable quadrat size to employ. A study of Tables 6 and 7 in the Appendix shows that the standard error of the sample mean and population mean increases as the ratio n_0/n_1 approaches unity. This is most likely to occur when the percentage absence is very small; in other words, when the quadrat size is such that it gives a percentage frequency of 90 to 100 per cent., the estimate of density is open to suspicion and the ratio between the first two frequency classes should be carefully examined. From the results for field observations given in Tables II and V it appears that, for a high degree of cover in grassland and herbaceous maritime communities, a quadrat of about 20 sq. cm. is suitable for estimating the density of the majority of species in the community.

IV. THE VALUES OF THE PARAMETERS IN RELATION TO QUADRAT SIZE

The parameter *m* has been defined as the mean number of cluster per quadrat, and $(1+\lambda)$ as the mean number of units per cluster; it is therefore of interest to both statisticians and ecologists to examine the change in the magnitude of the parameters with a change of quadrat size in order to see what the relation is between the biological cluster and the hypothetical cluster. For this purpose two sets of observations made with two different sizes of quadrat but within the same community are compared in Table V. The first series was made in September 1947 with quadrats of 20 sq. cm., and the second series in May 1948 with quadrats of 25 sq. cm.; in both series the quadrats were taken contiguously. The two series of observations were made on different patches of vegetation a few yards apart in a *Limonium* marsh community at Blakeney Point, Norfolk. In Fig. 3 the change in the parameters for nine of the most frequent species is shown graphically.

Table V

Comparison of Thomas's Parameters for Quadrats of Different Sizes

Species.	*Limonium* marsh, September 1947. 100 quadrats. Quadrat size: 20 sq. cm.							*Limonium* marsh, May 1948. 195 quadrats. Quadrat size: 25 sq. cm.						
	Frequency class.		Coeff. of disper-sion.	Thomas's estimated parameters.		Density.		Frequency class.		Coeff. of disper-sion.	Thomas's estimated parameters.		Density.	
	0.	1.		*m*.	(1+λ).	Ob-served.	Calcu-lated.	0.	1.		*m*.	(1+λ).	Ob-served.	Calcu-lated.
Obione portulaccoides .	97	3	0·97	0·03	0·983	0·03	0·030	191	4	0·979	0·021	0·989	0·020	0·020
Spergularia marginata .	97	3	0·97	0·03	0·983	0·03	0·030	192	3	0·984	0·016	1·008	0·015	0·016
Cochlearia officinalis .	91	7	0·34	0·094	1·202	0·11	0·113	160	19	4·621	0·198	1·510	0·467	0·299
Puccinellia maritima .	94	4	3·63	0·069	1·388	0·13	0·086	63	4	14·261	1·130	2·827	6·646	3·827
Festuca sp. . . .	82	4	4·98	0·198	2·403	0·67	0·591	172	9	4·030	0·125	1·875	0·343	0·235
Aster tripolium . .	76	13	2·23	0·274	1·473	0·43	0·404	157	28	1·575	0·217	1·412	0·277	0·306
Armeria maritima . .	57	6	3·39	0·562	2·675	1·58	1·504	80	28	2·959	0·893	1·937	1·995	1·730
Suaeda maritima . .	60	18	1·50	0·511	1·532	0·68	0·783	22	32	3·278	2·182	1·406	4·117	3·068
Triglochin maritimum .	39	23	1·50	0·941	1·468	1·32	1·382	26	14	2·389	2·015	2·320	4·123	4·674
Plantago maritima . .	12	8	2·85	2·120	2·158	5·05	4·558	49	4	10·735	1·379	3·827	9·041	5·276
Limonium vulgare . .	15	17	1·147	1·897	1·515	2·31	2·863	21	25	1·906	2·228	1·627	3·385	3·625
Salicornia stricta . .	4	3	3·90	3·219	2·428	6·99	7·814	5	6	4·527	3·664	2·116	8·282	7·753

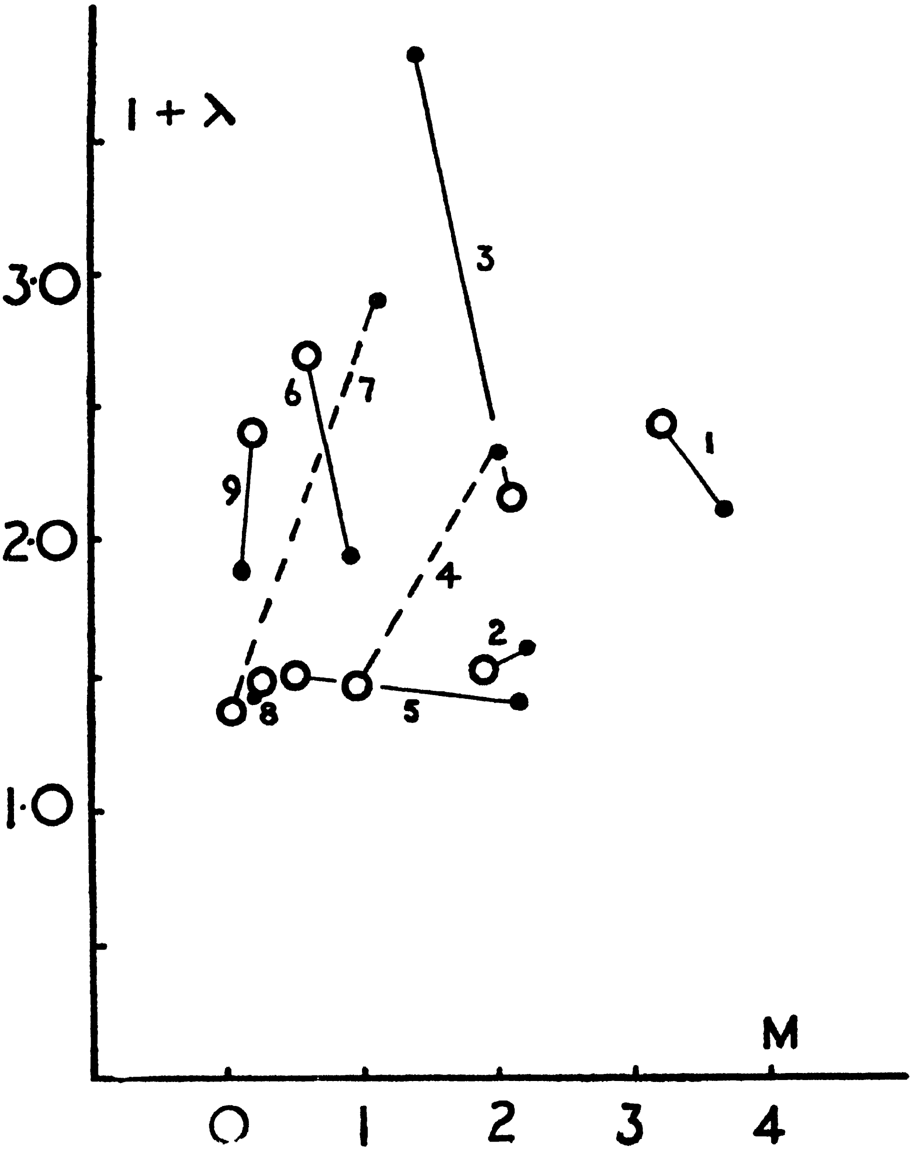

FIG. 3. Comparison of the estimated parameters of Thomas's series for pairs of similar species when observed with quadrats of different sizes.

○ = 20 sq. cm. quadrat. ● = 25 sq. cm. quadrat. *m* = estimated mean number of clusters per quadrat. $(1 + \lambda)$ = estimated mean number of individuals per cluster.

1. *Salicornia stricta.*	6. *Armeria maritima.*
2. *Limonium vulgare.*	7. *Puccinellia maritima.*
3. *Plantago maritima.*	8. *Aster tripolium.*
4. *Triglochin maritimum.*	9. *Festuca* sp.
5. *Suaeda maritima.*	

For the purpose of the present analysis the species may be considered in three sections according to the change in the absolute density. Firstly, there are the species in which the number of shoots per quadrat is less than would be expected for an increase in quadrat size of 5 sq. cm., provided the density remained constant over the whole area of the community. Under this category we have *Obione*, *Spergularia*, *Festuca*, and *Aster* and we see that, with an increase in quadrat size but a decrease in density, there is a decrease in the number of clusters per quadrat (m) while the cluster size remains more or less constant.

Secondly, there are the species in which the increase in the absolute density is almost exactly proportional to the increase in quadrat size, as in the case of *Limonium*, *Armeria*, and *Salicornia*. The parameter m shows a slight increase in all three species with the increase in quadrat size, while the parameter $(1+\lambda)$ remains more or less constant for *Limonium* and *Salicornia* but decreases for *Armeria*.

Finally, there is the group of species for which the density shows an unexpectedly great increase for an increase of 5 sq. cm. in the sample area. For *Suaeda* there is an increase in cluster number but a slight decrease in cluster size, while for *Cochlearia* and *Triglochin* there is an increase in both cluster number and cluster size. On the other hand, *Plantago* shows a decrease in cluster number but an increase in cluster size.

If we consider biological clusters, as, for example, the cluster of individuals which arise when seeds germinate in close proximity to the parent plant or the cluster of rooted shoots which grow adventitiously from a single parent, and compare them with the hypothetical clusters as defined by Thomas's series, then a possible explanation can be offered for the change in parameters with a change in quadrat size. If the hypothetical clusters were proportional to the biological clusters, and the quadrat was larger than the biological cluster then, with increase in quadrat size, we would expect an increase in the average number of clusters per quadrat (m) while the number of units per cluster $(1+\lambda)$ remained more or less constant. Except for *Plantago* this is more or less true for all species in Table V which show an increase in density with increase in quadrat size. For all these species there is an increase in the value of m, while the value of $(1+\lambda)$ is slightly above or below the expected value. If, on the other hand, the quadrat size is less than that of the biological cluster, then it is possible that the method of analysis picks out aggregation or clustering within the large natural cluster and that any increase in the size of the quadrat to include the whole natural cluster would reduce the number of clusters per quadrat but would increase the number of units per cluster. If this assumption is correct, then the change in the parameters for *Plantago* can be satisfactorily explained, and we would conclude that 20 sq. cm. was not sufficiently large to include the average *Plantago* cluster.

V. CONCLUSION

It will be seen that the present paper offers a direct mean for estimating the number of individuals per unit area for heterogeneous populations in which the variance is significantly greater than the mean. This avoids the necessity of applying a correction, as suggested by Ashby (1935), when the assumption is made that the individuals are distributed at random. Only two frequency classes are used in the estimation, and in this way the method is simpler than that suggested by Cole (1947).

The relationship between the natural clusters and the hypothetical clusters is not yet clear. In the Contagious Series Neyman defined the parameters in terms of the biological constants of the entomological problem to which his attention had been drawn, and in this ideal form the parameters are related to the spatial limits of these constants. In applying this series to plant populations it was assumed that the parameters were proportional to the number of clusters per unit area and the number of individuals per cluster. For practical purposes this is stated more simply in Thomas's series which does not attempt to define the parameters in terms of the size of the plants or of the natural clusters. Thus, while the general hypothesis of contagion appears to be acceptable, we cannot yet offer a direct relation between the parameters for clustering and the biological cluster until we know more exactly the size of the clusters of individuals for different species.

VI. SUMMARY

Data for the number of individuals per unit area for 27 species from the observation of 9,340 quadrats is discussed.

It is shown that Thomas's Double Poisson series will give a good estimate of the density of individuals in heterogeneous populations in which the variance is significantly greater than the mean. The estimate is given by the two parameters of the series which can be obtained by observation from the number of quadrats with no individuals and the number of quadrats with only one individual where

$$P\{0\} = e^{-m}$$

and

$$P\{1\} = e^{-(m+\lambda)},$$

m is the estimated mean number of clusters per quadrat and λ is the estimated number of units per cluster additional to the first unit. The estimated mean number of individuals per quadrat is equal to $m(1+\lambda)$.

Tables for the estimated parameters and the estimated mean density together with their standard errors are given in the Appendix.

The author wishes to thank Miss Marjorie Thomas of the Statistics Department, University College, London, for permission to reproduce the Tables given in the Appendix.

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APPENDIX

THOMAS'S DOUBLE POISSON SERIES

A. *The Estimation of the Parameters and their Errors*

The parameters m and λ of Thomas's series are obtained from the first and second moments of the sample distribution, or they may be estimated from the first two frequency classes of the distribution. The derivation of the estimated parameters by means of the method of maximum likelihood is given by Thomas (1949). Tables 1 to 4 give the estimated values of m and $(1+\lambda)$ and their standard errors according to this method. The appropriate equations and examples illustrating the use of the tables are given below.

Estimate of m:

$$e^{-\hat{m}} = n_0/N, \qquad \text{(Table 1)}$$

where $\hat{m}$ is the estimated mean number of clusters per quadrat,

N is the total number of quadrats,

n_0 is the number of quadrats with no individuals.

Standard error of $\hat{m}$:

$$\sigma_{\hat{m}}^2 = \frac{1}{N}\left(\frac{1-e^{-m}}{e^{-m}}\right)$$

$$= \frac{1}{N}\left(\frac{1}{n_0/N}-1\right). \qquad \text{(Table 1)}$$

Estimate of $(1+\lambda)$:

$$\hat{m}e^{-\hat{\lambda}} = n_1/n_0, \qquad \text{(Table 2)}$$

where $(1+\hat{\lambda})$ is the estimated mean number of individuals per cluster, and n_1 is the number of quadrats with only one individual.

Estimate of standard error of $\hat{\lambda}$:

$$\sigma_{\hat{\lambda}}^2 = \frac{1}{N}\left[\frac{1}{n_1/N}-\frac{1}{\hat{m}^2}+\frac{(1-\hat{m})^2}{\hat{m}^2 n_0/N}\right]. \qquad \text{(Table 3)}$$

The tables are calculated for $N = 100$ quadrats. The estimates of m and $(1+\lambda)$ are true for any number of quadrats, but the estimates of their standard errors are not. The error for any number of quadrats N' is obtained by multiplying the tabulated values by $10/\sqrt{N'}$.

The use of the tables is illustrated in the following example.

For *Armeria maritima* $N = 100$; $n_0 = 57$; $n_1 = 6$.

By interpolation from Table 1.

When $n_0/N = 0{\cdot}57$, then $\hat{m} = 0{\cdot}565$ and $\sigma_{\hat{m}} = 0{\cdot}087$ or an error of 15·4 per cent.

From Tables 2, 3, and 4.

When $n_0/N = 0{\cdot}57$ and $n_1/N = 0{\cdot}06$, then $(1+\lambda) = 2{\cdot}612$; $\sigma_{\hat{\lambda}} = 0{\cdot}395$ or 15·1 per cent.

TABLE 1

Estimated Values of m, $\sigma_{\hat{m}}$, and $\sigma_{\hat{m}}/\hat{m}$

n_0/N	0·05	0·10	0·20	0·30	0·40	0·50	0·60	0·70	0·80	0·90
$\hat{m}$	2·996	2·303	1·609	1·204	0·916	0·693	0·511	0·357	0·223	0·105
$\sigma_{\hat{m}}$	0·436	0·300	0·200	0·153	0·122	0·100	0·082	0·065	0·050	0·033
$\sigma_{\hat{m}}/\hat{m}$	0·145	0·130	0·124	0·127	0·134	0·144	0·160	0·183	0·224	0·317

TABLE 2

Estimated Values of $(1+\lambda)$

$n_1/N \diagdown n_0/N$	0·05	0·10	0·20	0·30	0·40	0·50	0·60	0·70	0 80	0·90
0·05	2·097	2·527	2·862	2·977	2·992	2·936	2·814	2·609	2·272	1·637
0·10	1·404	1·834	2·169	2·284	2·299	2·243	2·120	1·916	1·579	—
0·20	—	1·141	1·476	1·591	1·605	1·550	1·427	1·223	—	—
0·30	—	—	1·070	1·186	1·200	1·144	1·022	—	—	—

TABLE 3

Estimated Values of $\sigma_{\hat{\lambda}}$

$n_1/N \diagdown n_0/N$	0·05	0·10	0·20	0·30	0·40	0·50	0·60	0·70	0·80	0·90
0·05	0·536	0·480	0·451	0·441	0·434	0·428	0·421	0·410	0·388	0·317
0·10	0·433	0·361	0·321	0·307	0·297	0·288	0·277	0·261	0·225	—
0·20	—	0·283	0·231	0·210	0·196	0·182	0·164	0·134	—	—
0·30	—	—	0·191	0·166	0·147	0·128	0·101	—	—	—

TABLE 4

Estimated Values of $\sigma_{\hat{\lambda}}/(1+)\hat{\lambda}$

$n_1/N \diagdown n_0/N$	0·05	0·10	0·20	0·30	0·40	0·50	0·60	0·70	0·80	0·90
0·05	0·256	0·190	0·158	0·148	0·145	0·146	0·149	0·157	0·171	0·193
0·10	0·309	0·197	0·148	0·134	0·129	0·129	0·131	0·136	0·143	—
0·20	—	0·248	0·156	0·132	0·122	0·117	0·115	0·109	—	—
0·30	—	—	0·179	0·140	0·123	0·112	0·099	—	—	—

B. *The Estimated Mean Number of Individuals per Quadrat*

In this case we wish to draw inferences respecting the total population from the observation of a number of small samples. We shall therefore distinguish between the sample mean and the theoretical population mean which is estimated from the first and second frequency classes of the sample distribution.

Values for the estimated mean $M = m(1+\lambda)$ are given in Table 8.

The standard error of the estimate of M given by the sample mean for $N =$ 100 is

$$\sqrt{\{m(1+3\lambda+\lambda^2)/N\}} = \sqrt{(\mu_2/N)}, \qquad \text{(Table 6)}$$

where μ_2 is the second moment of the population.

The standard error of the estimated population mean, M, found by the maximum likelihood method, is given by

$$\sigma_M^2 = \frac{1}{N}\left[\frac{(\hat{m}-\hat{\lambda}-2)^2}{n_0/N}+\frac{\hat{m}^2}{n_1/N}-(\hat{\lambda}+2)^2\right] \qquad \text{(Table 7)}$$

The comparative reliability of the two estimates can then be judged by the ratio of the two standard errors. This is done in Table 8, which gives values for the ratio $\sqrt{\mu_2}/\sigma_M\sqrt{N}$. It will be seen from values on the left-hand side of Table 8, where n_0/n_1 approaches unity, that the ratio of the two standard errors is smallest, indicating that the maximum likelihood method gives an unreliable estimate of the population mean. Values on the right-hand side of Table 8 are high showing that the loss of accuracy due to the maximum likelihood method is small.

As an example we may consider the same data for *Armeria maritima* as was used before.

From Table 5 for the estimated mean M.

When $n_0/N =$ 0·57 and $n_1/N =$ 0·06 then $M =$ 1·537 (the observed sample mean being 1·58).

From Table 8 the ratio of the standard errors for $n_0/N =$ 0·57 and $n_1/N =$ 0·06 is 0·75, which indicates that the maximum likelihood method is satisfactory.

TABLE 5

Estimated Values of Mean $M = m(1+\lambda)$

n_1/N \ n_0/N	0·05	0·10	0·20	0·30	0·40	0·50	0·60	0·70	0·80	0·90
0·05	6·283	5·819	4·606	3·585	2·741	2·035	1·437	0·931	0·507	0·172
0·10	4·206	4·223	3·491	2·750	2·106	1·554	1·083	0·683	0·352	—
0·20	—	2·627	2·375	1·916	1·471	1·074	0·729	0·436	—	—
0·30	—	—	1·722	1·427	1·099	0·793	0·522	—	—	—

TABLE 6

Standard Error of Estimate of M given by Sample Mean for N = 100

n_1/N \ n_0/N	0·05	0·10	0·20	0·30	0·40	0·50	0·60	0·70	0·80	0·90
0·05	0·406	0·427	0·402	0·361	0·317	0·270	0·223	0·173	0·120	0·059
0·10	0·267	0·311	0·307	0·280	0·246	0·209	0·169	0·128	0·083	—
0·20	—	0·182	0·207	0·194	0·171	0·143	0·112	0·078	—	—
0·30	—	—	0·140	0·138	0·123	0·100	0·074	—	—	—

TABLE 7

Standard Error of Maximum Likelihood Estimate of M for N = 100

n_1/N \ n_0/N	0·05	0·10	0·20	0·30	0·40	0·50	0·60	0·70	0·80	0·90
0·05	1·304	1·042	0·789	0·623	0·495	0·389	0·298	0·215	0·138	0·062
0·10	0·954	0·691	0·529	0·426	0·342	0·270	0·205	0·146	0·089	—
0·20	—	0·471	0·325	0·264	0·213	0·167	0·124	0·082	—	—
0·30	—	—	0·233	0·181	0·144	0·110	0·077	—	—	—

TABLE 8

Ratio of Two Standard Errors

n_1/N \ n_0/N	0·05	0·10	0·20	0·30	0·40	0·50	0·60	0·70	0·80	0·90
0·05	0·311	0·410	0·510	0·580	0·640	0·695	0·749	0·805	0·867	0·947
0·10	0·280	0·450	0·581	0·657	0·718	0·772	0·824	0·877	0·935	—
0·20	—	0·387	0·635	0·736	0·801	0·855	0·906	0·956	—	—
0·30	—	—	0·601	0·766	0·850	0·911	0·963	—	—	—

Cell-multiplication and Cell-enlargement in the Development of the Flesh of the Apple Fruit

BY

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With seven Figures in the Text

INTRODUCTION

AT maturity the apple fruit may be considered as the result of a sequence of development in which a cellular structure has been arrived at by three recognizable processes, namely, cell-division, cell-enlargement, and cell-differentiation. The main bulk of the fruit consists of a parenchymatous tissue forming the flesh, of which anatomically the cortex constitutes the greater proportion by volume.[1] The cells of the cortex comprise a spongy tissue; these cells are large, thin-walled, with a thin lining of cytoplasm and a large vacuole. The tissue is homogeneous, and the main interest in the analysis of development presented here lies in the direction of determining how cell-division and consequent multiplication of cells on the one hand, and cell-enlargement on the other hand, proceed at different stages of the ontogeny of the fruit. It can readily be seen that both the size of the fruit and the structure of its flesh will be materially affected by the periodicity and intensity of these processes.

In a previous paper (Smith, 1940) the author described the results of an investigation of the structure of the cortex of the mature apple in a number of different varieties in terms of cell-size in relation to fruit-weight. It was there shown that larger apples had both more and larger cells than smaller apples. Furthermore, the size of cell in earlier-maturing varieties was smaller, the rate of respiration higher, and the keeping quality poorer than in later-maturing varieties.

The present paper brings together the results of some studies of the course of development in the cortical region of the growing fruit in a number of varieties with widely differing characteristics, in an attempt to evaluate the parts played by the two formative processes, cell-multiplication and cell-enlargement. These studies have been extended back into the 'pre-fruit' stages of development.

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[1] The percentages by weight of peel, cortex, pith, and carpellary tissues as determined for the Newton Wonder apple are approximately as follows:

Wt. of fruit in g. .	94·2	118·1	143·9	168·4	194·5
Peel . . .	5·1	5·0	4·3	4·1	4·0
Cortex . .	91·7	91·9	92·7	92·8	93·1
Pith and carpels .	3·2	3·1	3·0	3·1	2·9

[Annals of Botany, N.S. Vol. XIV, No. 53, January 1950.]

MATERIAL AND METHODS

The following varieties were selected for study:

Early Victoria (*Emneth Early*). An early-maturing culinary apple growing to relatively large size.

Beauty of Bath. An early-maturing dessert apple, attaining only relatively small size at maturity.

Worcester Pearmain. A second-early dessert apple growing to moderate size.

Lord Derby. A late mid-season culinary apple growing to large size.

Cox's Orange Pippin. A late dessert variety of small to moderate size.

Newton Wonder. A late culinary apple of moderate to large size.

Bramley's Seedling. A late culinary apple of large size.

King Edward VII. A late culinary apple of small to moderate size with characteristic late blossoming.

Court Pendu Plat. A very late dessert variety; very late blossoming. Small.

Collection of buds, blossoms, and developing fruits was made from the commencement of growth in early spring up to full maturity of the fruit, the material being fixed and stored in formalin-acetic-alcohol. The intervention of the war prevented full use being made of the material and consequently some of the data presented here are fragmentary. Such material as it was possible to examine was handled as follows. Buds were cut in half longitudinally using a sharp hollow-ground razor, washed in tap-water, and stained lightly with Delafield's haematoxylin. One-half of each bud was then mounted in glycerin on the cover-glass of a Leroux Press-chamber and examined with vertical illumination by means of the Leitz Ultrapak microscope using an immersion cap. In any one bud the king-flower was usually to be found sectioned through the floral axis together with one or more lateral flowers similarly sectioned. Using a drawing eye-piece and low-power objective, the outline of the flower and its developing organs was traced. With a higher-power objective it was possible to trace the outlines of cells lying in the cortical region between the primary vascular strands and the hypodermis; samples of 30 cells were drawn at a position approximately on a level with the middle of the developing ovary. The following measurements were recorded for 6 king-flowers and 6 lateral flowers of each lot examined (Fig. 1). The position of line *aa′* was determined by contingency with the boundary of the tissue at the base of the stamens and styles. The line *bb′* was drawn parallel to *aa′* at a distance determined by the centres of developing ovules, or before they appeared, at what was judged to be the centre of the ovary. *ab* and *a′b′* were drawn at right angles to *aa′*, *bb′*, intersecting the outline of the outer surface of the flower at a point half-way between *aa′* and *bb′*. The distance between the lines *ab* and *a′b′* (Fig. 1) was measured and designated $2r$. The distance between the lines *aa′* and *bb′* was designated l. An arbitrary rule for estimating the volume of the portion of the developing fruit which would later comprise the main bulk of fleshy tissue was to calculate the volume V of a cylinder, where $V = 2\pi r^2 l$.

When the flowers had become sufficiently developed (at the 'burst' stage[1]) they were sectioned individually, then treated in the same way. After fertilization, the fruitlets were soon sufficiently developed to cut thin sections of the cortical tissue by hand. These sections were washed, stained in Delafield's haematoxylin, and mounted on a slide in 50 per cent. glycerin in water. Drawings were made by means of a projection microscope, and the mean area of cell determined by means of a planimeter for samples of 100 cells taken from sections at opposite sides of each apple. Cell-volume was calculated on the arbitrary assumption that the actual volume was not very different from that of a sphere with equivalent cross-section in a plane through the centre. Thus, if mean area of cell $= A$, then volume $v_c = 1{\cdot}33\sqrt{(A^3/\pi)}$. In the more developed stages, owing to the gradient in size of cell between the outer and inner cortex (Smith, 1935), the precaution was taken of sampling at a point at a distance from the skin of approximately $\frac{1}{14}$th of the fruit diameter, in the equatorial plane.[2] Fruit-size was determined in the post-pollination stages by weighing, care being taken when the fruits were small to trim the calyx and pedicel.

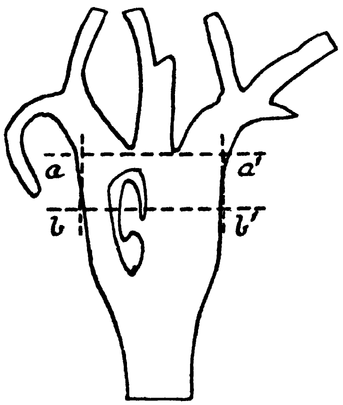

FIG. 1. Flower of the apple showing method of measurement in estimating volume during development.

DEVELOPMENT UP TO POLLINATION

Bijhouwer (1924) has traced in some detail the initiation of the floral primordia in the mixed bud and their development until early autumn, when he reported a stage of dormancy, which he attributed to prevailing low temperatures lasting until the spring. The purpose of the present investigation was to trace the subsequent development up to and including pollination and the setting of the fruit. The following recognizable stages are referred to in subsequent graphs.

Stage A. At the earliest stage examined (February 23) it was evident that growth had not yet recommenced, the buds being tight-closed. The five carpels were the least developed of the floral parts, though the cavity bounded by the bases of these carpels clearly showed the position of the future ovaries.

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[1] The terms used here to describe the stages of fruit-bud development are those used in Fruit Bud Development and illustrated by a series of charts, Bull. No. 137, Min. of Agr., H.M.S.O.

[2] Approximately 75 per cent. by volume of the fruit is contained in a shell of tissue extending from the surface to a depth of $\frac{1}{7}$th the diameter of the fruit.

Stage B. A further advance in growth of all the parts occurred prior to any external change in the appearance of the bud. The carpels began to infold at the margins as described by Kraus (1913).

Stage C. There was a further development of sepals, petals, and stamens by the time the buds began to break and the green bracts had begun to show between the scales.

Stage D. The ovules had begun to develop on the placentae formed by the infolded edges of the carpels, when the individual flowers were discernible emerging from the sheath of scales.

Stage E. By the time of the so-called 'pink-bud' stage the sepals had developed greatly and had begun to bend away from the floral axis, the styles had extended upwards, and the ovules increased in size.

Stage F. At full bloom the bases of the sepals formed a platform bearing the petals and stamens and the carpels sunk in the surrounding receptacular tissue formed the five fully developed locules.[1]

The main interest in this investigation centred round the increase in magnitude of the dimensions $2r$, l, and V as measures of the progressive development of the fleshy portion of the future fruit. In Fig. 2, therefore, is shown in a series of graphs for the Early Victoria and Bramley's Seedling varieties the values of $2r$ and l for both king and lateral flowers over the period of observation during the spring of 1939. In Fig. 3*a* and 3*b* the calculated volume V is similarly shown.

Dimension 'l'

Until the beginning of March there was scarcely any appreciable change in this dimension, but from then onwards there was an increase in magnitude that became more and more rapid until the latter part of April, when for a short period, corresponding to full bloom, enlargement ceased. It will be seen that the king flowers were of larger average dimension throughout.

Dimension '2r'

The magnitude of this dimension throughout followed the same general tendency as l.

The relation between 'l' and 'r'

It is of some interest to relate l and r for the different varieties, which are of different shape at maturity. This has been done in Table I, where the ratio l/r at the cessation of enlargement has been calculated from values extracted from the development curves and tabulated with characteristic shape of the variety, 'conical', 'tall', or 'flat'.

It will be seen from this that there is some suggestion of a tendency for

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[1] With the relative merits of the axial and appendicular theories of the origin of the apple fruit we are not concerned here. As a basis for description the terms appropriate to the axial theory are employed.

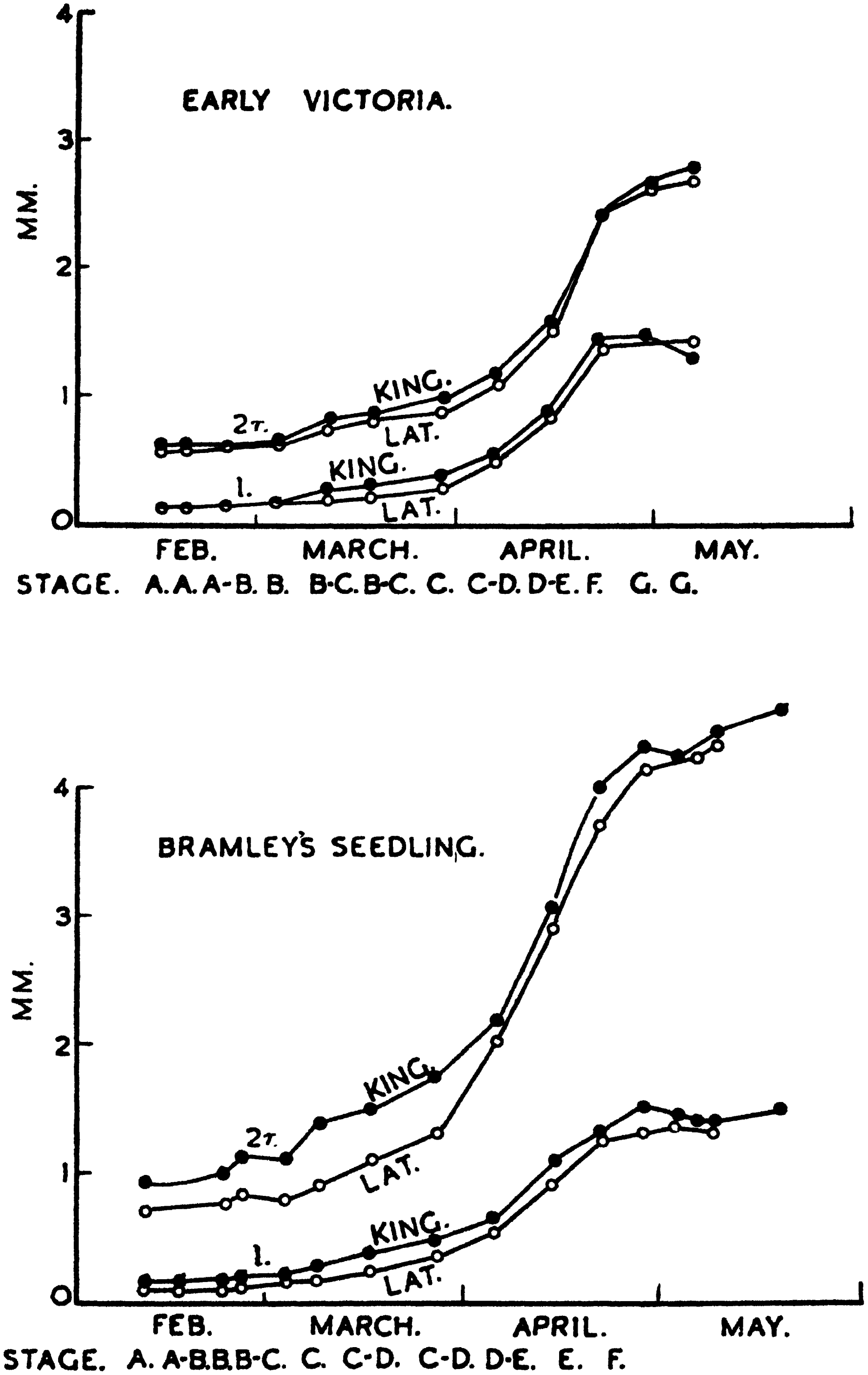

Fig. 2. Change of dimensions *l* and *2r* during pre-pollination phase of flower development in Bramley's Seedling and Early Victoria varieties of apple. 1939.

King. = King flower. Lat. = Lateral flower.

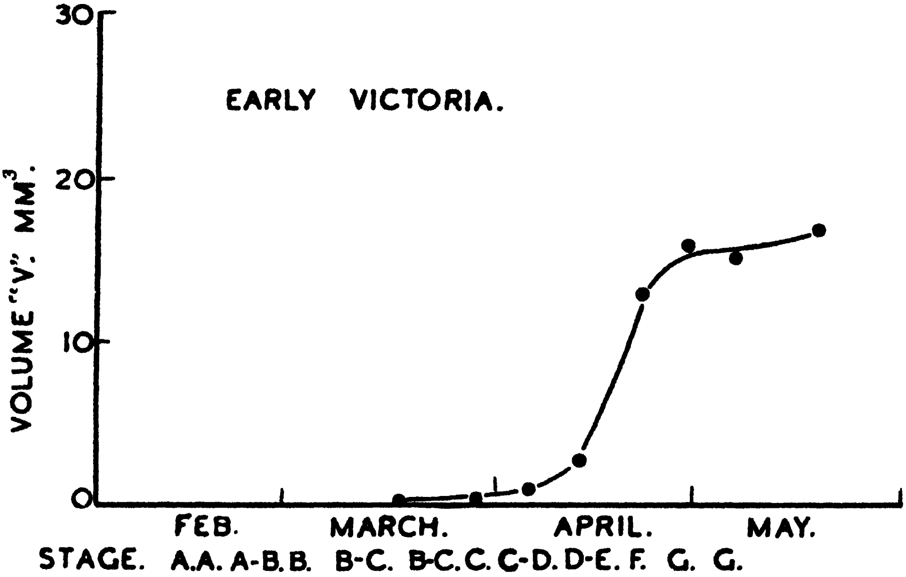

FIG. 3*a*. Changes in volume *V* during pre-pollination stages of development of Early Victoria variety of apple. 1939.

conical shape at maturity, as opposed to flat shape, to be foreshadowed in the flower, the flat varieties having distinctly lower values of *l*/*r* than the conical or tall varieties.

TABLE I

The Relation of the Dimensions 'l' and 'r' at Full Bloom to Varietal Shape at Maturity

Variety	*l*/*r*	Fruit-shape
Early Victoria	0·54	Conical
Worcester Pearmain	0·50	Conical
Cox's Orange Pippin	0·47	Somewhat conical
Lord Derby	0·46	Tall
Newton Wonder	0·40	Somewhat flattened
King Edward VII	0·37	Slightly conical
Beauty of Bath	0·37	Flat
Court Pendu Plat	0·36	Very flat
Bramley's Seedling	0·33	Distinctly flat

Dimension 'V'

In Fig. 3*a*, *b*, the values for *V* (for king and lateral flowers combined) have been plotted against time. It will be seen from these graphs that there is in general a phase of gradually increasing rate of development from the beginning of March until the latter part of April, corresponding to the time of full bloom. The curves are particularly steep during the week before full bloom, and their form suggests a quite abrupt cessation of growth in volume when the flower opens, followed again by a rapid increase, the stimulus for which may be attributed to pollination.

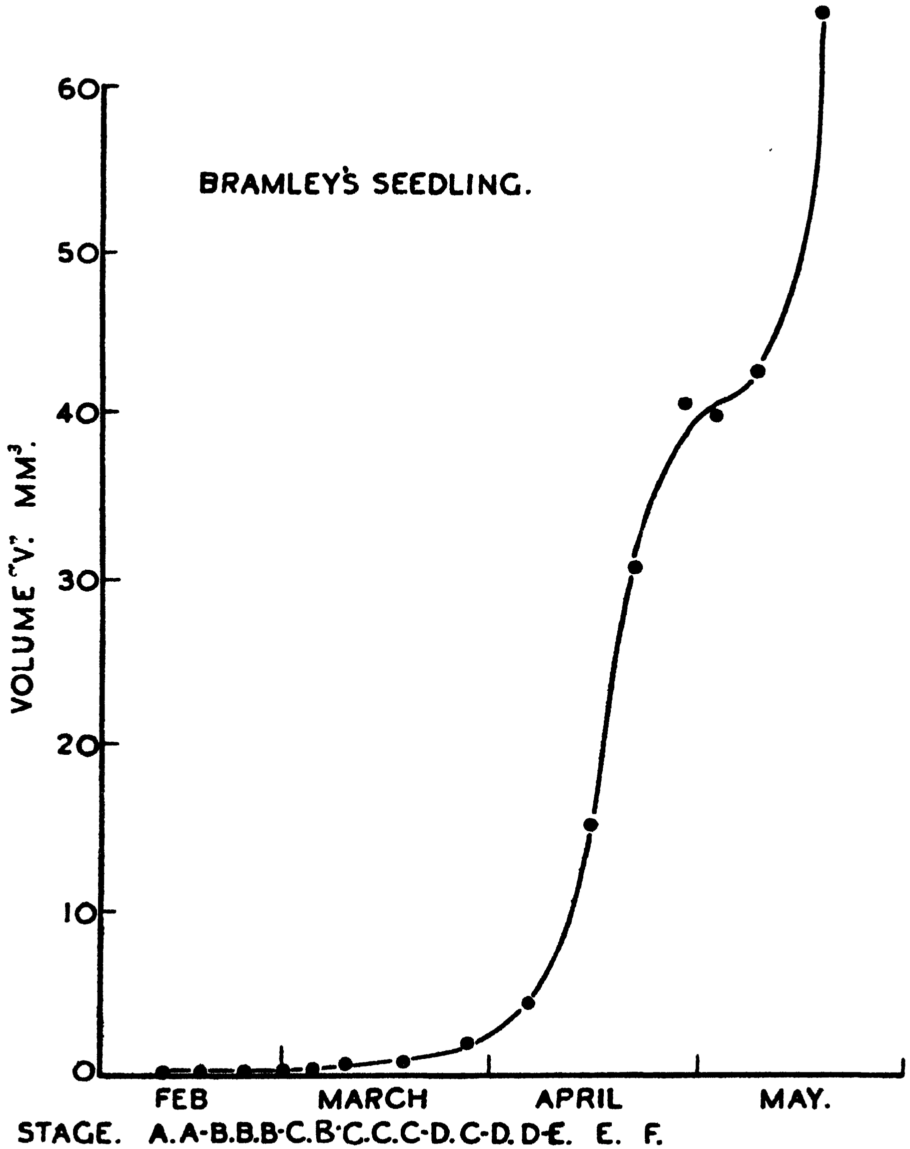

Fig. 3*b*. Changes in volume V during pre-pollination stages of development of Bramley's Seedling variety of apple. 1939.

Cell-multiplication and Cell-enlargement before Pollination

The data for cell-size in the pre-fruit stages are unfortunately incomplete. The values for cell volume v_c and calculated number N of cells in volume V are plotted against time in Figs. 4 and 5 for the varieties Early Victoria and Bramley's Seedling only. It will be seen that the values are given for king and lateral flowers combined, and that for each variety there are two curves, one for volume of cell calculated as a sphere from the measured sectional area, and the other for volume of cell calculated as a cube. The actual values will lie somewhere between the two, since at this stage the cells are isodiametric and rather more cubical in shape than later when with vacuolation they become

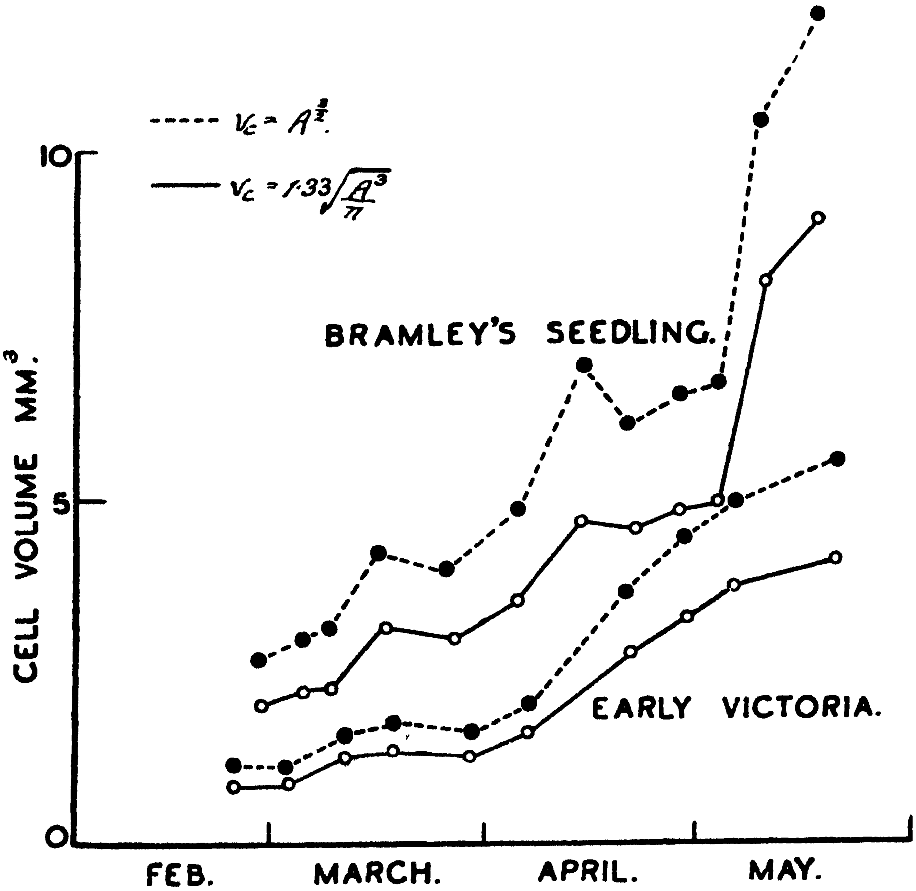

Fig. 4. Pre-pollination cell-volume v_c in Early Victoria and Bramley's Seedling varieties of apple. 1939.

more spherical. The number *N* of cells per volume *V* has been calculated by dividing the mean volume of cell v_c into *V*. Here again two sets of values are plotted, the one calculated on a cubical and the other on a spherical basis.

It will be seen that there is a slow but steady increase in cell-volume from the earliest stages onwards up to the time of full bloom, and later a rapid increase at the time of pollination. Multiplication of cells proceeded slowly at first, but at the end of March there was a rapid increase, corresponding to the steep climb in the values of volume *V*. This was followed by cessation of cell-multiplication towards the end of April. (The apparent decline is doubtless due to sampling error.) During the period in which the flower is fully open there appears to be no cell-multiplication; subsequently under the stimulus of pollination there is a tremendous spurt of activity.

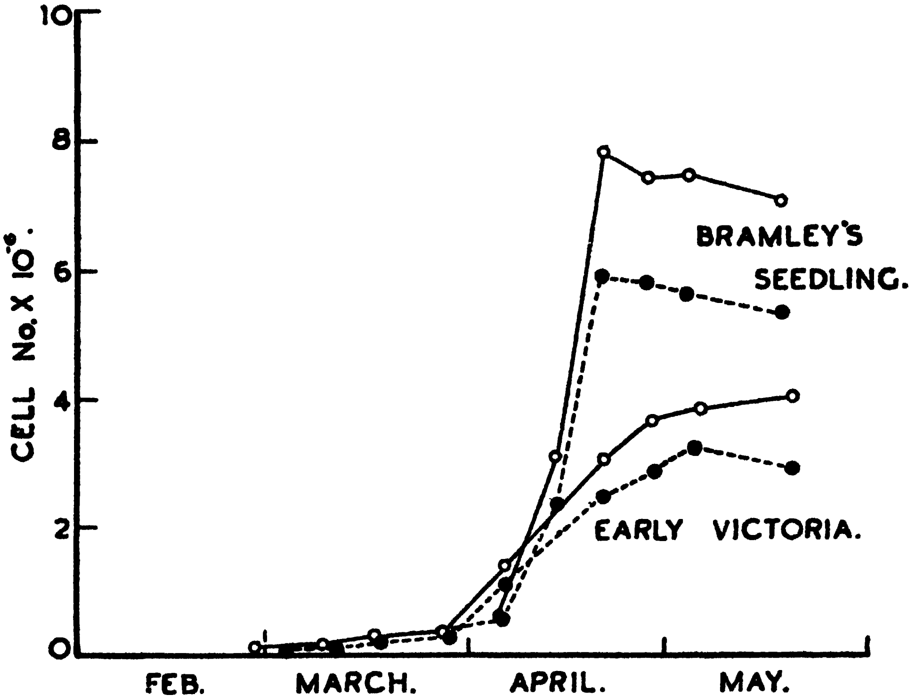

FIG. 5. Pre-pollination cell number N in Early Victoria and Bramley's Seedling varieties of apple. 1939.

The value for mean cell-volume v_c increased 4·3 times for Early Victoria and 2·5 times for Bramley's Seedling when the earliest stages examined were compared with the open-flower stage. The corresponding values for increase in number of cells were 31 times and 52 times. Thus from these observations it can be concluded that during the period of development, from the recommencement of bud-growth to the stage of fully opened flower, the dominant factor in determination of organ-size is the amount of cell-multiplication.

In Table II are shown values for organ-volume V, cell-volume v_c (mean of the values for cubical and spherical calculations), and calculated number of cells N for the nine varieties of apple examined at the stage of the fully opened flower. It will be seen that, with the exception of the varieties Lord Derby and Bramley's Seedling, these values are essentially comparable. The somewhat larger value of V for Lord Derby is attributable to a larger number of cells and of Bramley's Seedling to both greater number and larger size cells. The size of cell of the Bramley's Seedling at the commencement of bud-expansion was found to be nearly 3 times as great as that of the Early Victoria; the number of cells about 1·5 times as great. The size of the rudimentary flower was about 4 times as large as that of the Early Victoria. It is noteworthy that the Bramley's Seedling is the only triploid variety among those studied, the remainder being diploid.

TABLE II

Volume 'V', Volume of Cell 'v_c', and Number of Cells 'N' at the Stage of Fully Opened Flower

Variety.	Early Victoria.	Beauty of Bath.	Worcester Pearmain.	Lord Derby.	Cox's Orange Pippin.	Newton Wonder.	Bramley's Seedling.	King Edward VII.	Court Pendu Plat.
Volume V (mm.)	15·0	15·0	14·7	23·8	13·1	12·3	36·1	19·3	14·6
Cell-volume v_c (mm.)	4·3	4·5	3·8	4·0	3·5	4·5	5·8	4·5	3·5
Cell number N ($\times 10^{-6}$)	3·1	3·3	3·9	6·0	3·8	2·8	6·2	4·3	4·2

DEVELOPMENT AFTER POLLINATION

With the stimulus of pollination an impulse is given for the rapid development of the fruit. The morphological and anatomical changes taking place between pollination and the completion of development at maturity have been described by others (Kraus, 1913; Tetley, 1931; Tukey and Young, 1942). Here it is proposed to deal only with those aspects that relate to the analysis of cell-multiplication and cell-enlargement as processes in the formation of the main bulk of fleshy tissue.

While the method of sampling employed precludes classification of the smoothed curves from fruit-weight plotted against time in Fig. 6 as being representative growth-curves for the crops of the nine varieties of apple during the season 1939, the general form shows the relative increase in fruit-weight as between these varieties. The main point of interest lies in the relation between fruit-weight and cell-volume during development.

From this figure and from Fig. 7, in which the mean cell-volume is also plotted against time (the origin in each graph being at May 25), it will be seen that the two sets of curves are essentially similar in form, there being in each a phase of rapidly increasing rate of enlargement followed by a phase of more uniform rate, followed again in most varieties by a fall of rate with approaching maturity.

From the values for fruit-weight and mean cell-volume, the number of cells per apple can be estimated by the formula $N = \frac{W \times 10^3}{1{\cdot}1 \times v_c}$, where W is mean weight of fruit in grammes; v_c mean cell-volume in mm.^{3}, and 1·1 the value for specific gravity of the cell as calculated from measurements of the relative volume occupied by the cells in a standard volume of tissue (Smith, 1937).

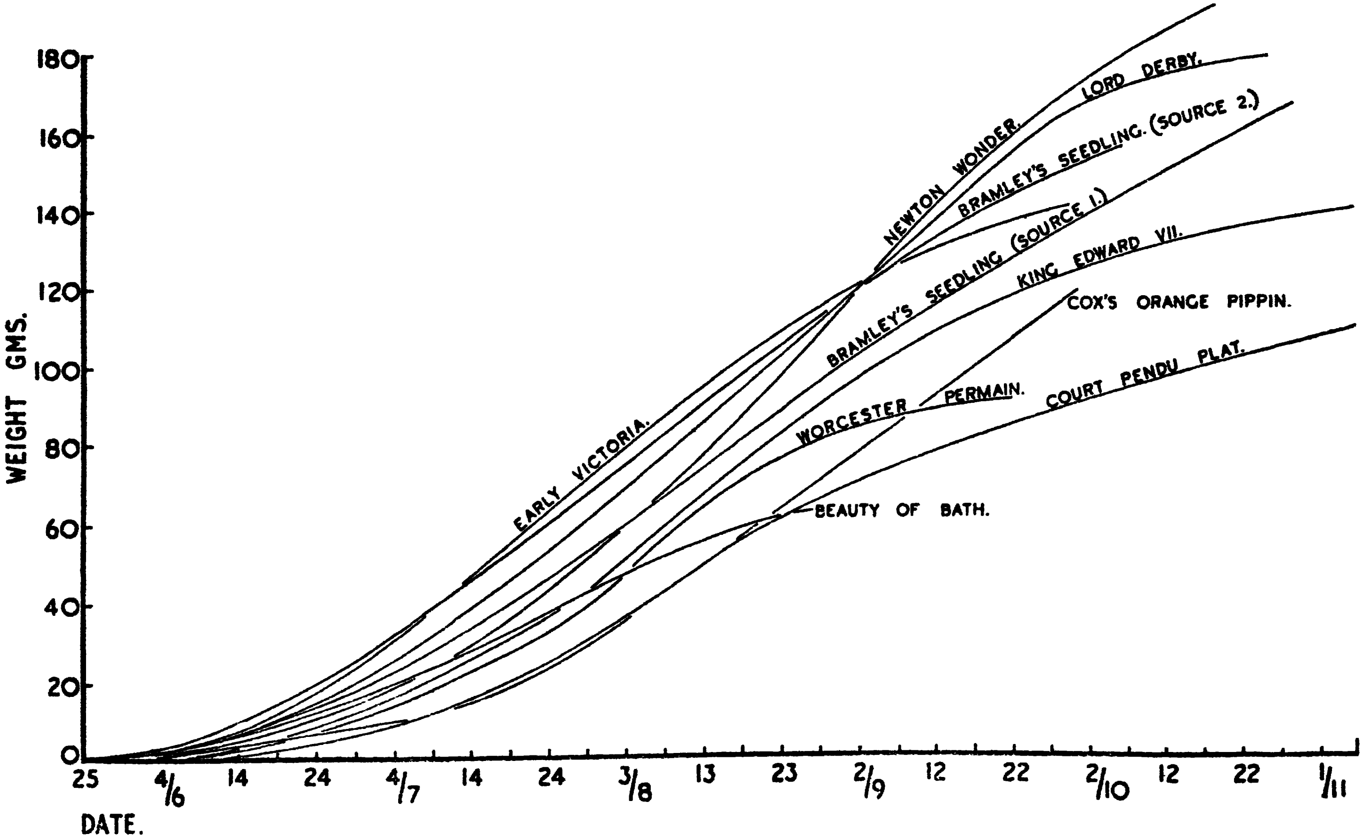

Fig. 6. Increase of fruit-weight during development of varieties of apple. 1939.

The values in Table III show that cell-multiplication is virtually completed by about the end of June, which is in agreement with the observations of Tetley (1931). Thereafter, increase in the volume of fruit is dependent upon increase in cell-volume together with increase in volume of the intercellular spaces.

TABLE III

Calculated Mean Number of Cells per Apple in Relation to Development

Variety. Early Victoria.		Worcester Pearmain.		Lord Derby.		Cox's Orange Pippin.		Newton Wonder.		King Edward VII.		Bramley's Seedling (Source 1).	
Date.	Calculated mean no. cells ($\times 10^{-6}$).	Date.	Calculated mean no. cells ($\times 10^{-6}$).	Date.	Calculated mean no. cells ($\times 10^{-6}$).	Date.	Calculated mean no. cells ($\times 10^{-6}$).	Date.	Calculated mean no. cells ($\times 10^{-6}$).	Date.	Calculated mean no. cells ($\times 10^{-6}$).	Date.	Calculated mean no. cells ($\times 10^{-6}$).
31/5	29·0	1/6	36·3	31/5	37·9	13/6	30·7	8/6	37·4	3/6	18·2	25/5	13·3
6/6	36·5	7/6	56·7	5/6	58·3	27/6	36·5	15/6	44·9	8/6	31·7	3/6	26·8
13/6	41·4	21/6	52·7	26/6	60·6	11/7	33·4	19/6	49·9	15/6	45·9	16/6	38·3
20/6	43·1	28/6	59·0	24/7	72·1	25/7	39·4	29/6	56·7	13/7	43·5	30/6	33·9
27/6	51·4	26/7	56·0	21/8	73·8	8/8	37·3	27/7	57·4	10/8	46·2	28/7	34·7
11/7	53·3	24/8	53·1	26/9	69·3	14/9	34·1	10/8	61·0	18/9	45·2	25/8	41·1
8/8	57·9	8/9	51·9	18/10	67·6	28/9	35·3	17/10	65·8	10/10	48·9	4/10	43·5
										3/11	42·2		

THE DETERMINATION OF FRUIT-SIZE

It may now be asked whether the characteristic mean size of fruit for a variety is determined primarily by number of cells, by degree of enlargement of cells, or equally by both factors. The varieties Beauty of Bath, Early Victoria, Worcester Pearmain, Lord Derby, Cox's Orange Pippin, Newton Wonder, King Edward VII, Bramley's Seedling, and Court Pendu Plat constitute a series progressive in season of maturation. While cultural and seasonal conditions exert a decided influence upon average fruit-size, these varieties can be classed as follows: *Small*, Beauty of Bath, Cox's Orange Pippin, and Court Pendu Plat; *Medium*, Early Victoria, Worcester Pearmain, King Edward VII; *Large*, Lord Derby, Newton Wonder, Bramley's Seedling.

Reference to Figs. 6 and 7 will show that the relation between increase in fruit-weight and cell-enlargement is by no means the same for each variety. For instance, though Lord Derby develops a large apple, the rate of cell-enlargement in this variety is only intermediate and the final size of cell inferior. The reason for this is to be found in an analysis of the calculated mean numbers of cells per fruit of mean weight collected together and arranged in descending order of magnitude for number of cells in Table IV. It is then seen that, if for the moment Bramley's Seedling is left out of account, the varieties Beauty of Bath, Cox's Orange Pippin, and Court Pendu Plat are the three lowest in order of number of cells. These are characteristically small-fruited varieties. The medium-fruited varieties King Edward VII, Early Victoria, and Worcester Pearmain are next in order of ascending

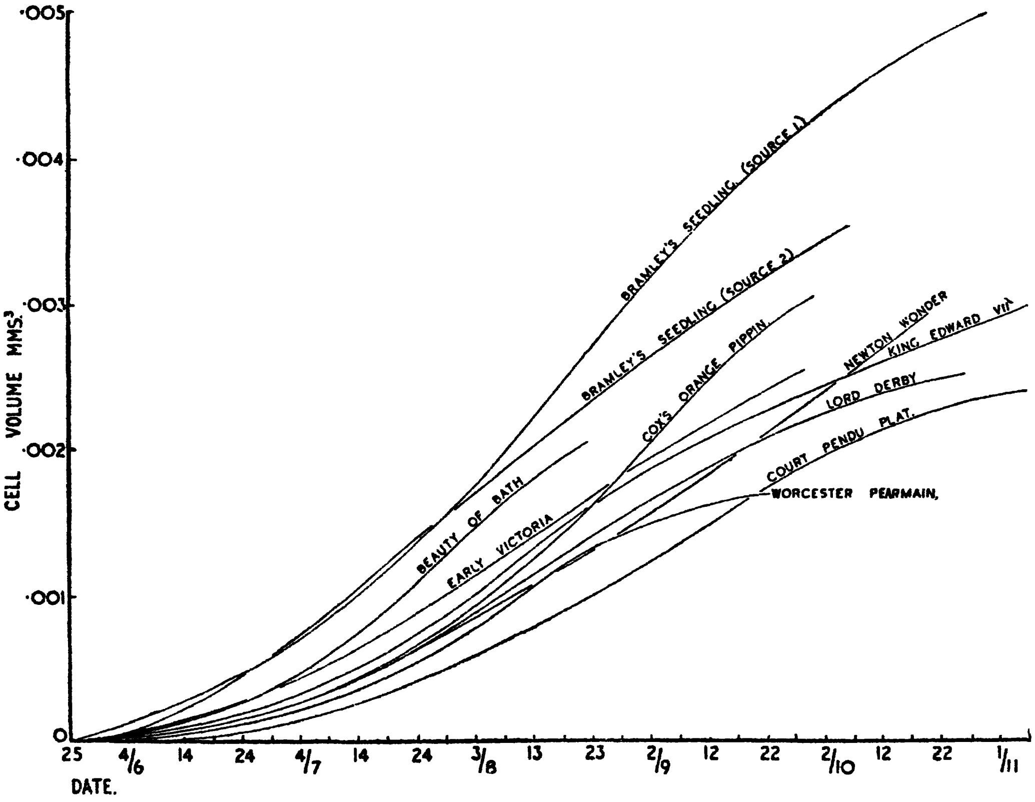

FIG. 7. Increase of mean cell-volume in flesh of fruit during development of varieties of apple. 1939.

magnitude, while Newton Wonder and Lord Derby have the largest number of cells. These two varieties are characteristically large-fruited.

TABLE IV

Mean Cell-volume and Mean Number of Cells in Mature Apple of Different Varieties

Variety.	Date.	Mean wt. (g.).	Mean cell-vol. (mm.^{3}).	Mean no. cells ($\times 10^{-6}$).
Lord Derby	Oct. 25	176	0·0024	67
Newton Wonder	Oct. 17	200	0·0029	62
Worcester Pearmain	Sept. 8	97	0·0016	55
Early Victoria	Sept. 27	142	0·0025	52
King Edward VII	Nov. 3	136	0·0028	44
Court Pendu Plat	Nov. 9	106	0·0023	42
Bramley's Seedling (Source 2)	Oct. 4	156	0·0037	38
Cox's Orange Pippin	Sept. 28	116	0·0030	35
Bramley's Seedling (Source 1)	Oct. 27	164	0·0048	31
Beauty of Bath	Aug. 22	60	0·0020	27

It is again found that the triploid Bramley's Seedling variety occupies an anomalous position since it is large-fruited but with a relatively small number of cells. If, however, we exclude this variety, there is evidence that characteristic size of fruit is determined primarily by the number of cells going to form it; variation in degree of cell-enlargement is of secondary importance in this connexion.

SEASONAL VARIATION

The mean individual fruit-weight at maturity of a variety for any given tree or set of trees may be expected to vary from year to year. It will, for instance, be higher in light-crop years than in heavy-crop years. The question therefore arises: Is high fruit-weight primarily associated with a large amount of cell-division, enhanced cell-enlargement, or with both?

In Table V the mean values for fruit-weight, cell-volume, and number of cells per apple at maturity have been tabulated for apples of the varieties Bramley's Seedling (two sources) and Early Victoria. From this table it can be seen that the mean weight per fruit varied from year to year. The mean volume of cell also varied from year to year, but not in strict relation to the size of fruit. A scrutiny of the column giving number of cells per fruit of mean weight reveals also that in each series there are both high and low values. Thus in the years 1939, 1940, 1941, and 1947 the low values appear, while in 1938 and 1942 high values (Bramley's Seedling). With Early Victoria low values occur in 1938 and 1940 and high values in 1939, 1941, and 1942.

It is probable that the mean fruit-weight tends to be in inverse relation to amount of crop borne, but no records are available of total crop borne by the trees in these years. There was, however, no extreme variation.

These results suggest that mean fruit-weight may be determined in one year primarily by amount of cell-multiplication, while in another by amount of cell-enlargement.

TABLE V

Mean Cell-volume and Mean Number of Cells per Apple in relation to Mean Fruit-weight in different Years.

Date picked.	Mean fruit-weight (g.).	Mean cell-volume (mm.^{3}).	No. cells per apple of mean weight ($\times 10^{-6}$).
	Bramley's Seedling (Source 1)		
Oct. 27, 1939	164	0·0048	31
Oct. 7, 1940	177	0·0040	40
Sept. 24, 1941	130	0·0030	39
Sept. 24, 1942	170	0·0033	47
Sept. 29, 1947	132	0·0030	40
	Bramley's Seedling (Source 2)		
Oct. 3, 1938	226	0·0042	49
Oct. 4, 1939	156	0·0037	38
Oct. 7, 1940	178	0·0040	40
Sept. 19, 1941	124	0·0031	36
Sept. 24, 1942	158	0·0029	50
	Early Victoria		
Aug. 19, 1938	106	0·0020	48
Aug. 1, 1939	107	0·0016	61
Aug. 21, 1940	94	0·0020	43
Aug. 16, 1941	101	0·0016	57
Aug. 24, 1942	87	0·0013	61

CONCLUSION

The main object of the investigations in progress is to elucidate the effect of various cultural factors in the growth of the apple fruit upon its anatomical structure and the effects of variation in structure upon senescent metabolism and keeping quality. This paper is limited to a presentation of the results of the analysis of cell-multiplication and cell-enlargement during the development of the apple fruit. It is not proposed to attempt to draw inferences at this stage.

SUMMARY

An analysis was made of cell-multiplication and cell-enlargement in the development of the flesh of the fruit of nine varieties of apple, varying both in characteristic fruit-size and season. The period from the recommencement of fruit-bud development in the spring to full maturity was covered.

During the period preceding pollination, in the development of the tissues which form the basis for the future flesh of the fruit, the cells to some extent enlarged, but the main increase in bulk of tissue was due to multiplication of cells. Development ceased abruptly with the opening of the flower.

Following pollination, the rate of increase in amount of flesh and in size

of cell climbed steadily during the early phase of development. Cell-multiplication virtually ceased to have significance for the development of the flesh by the end of June, by which time a more or less steady rate of increase in weight was associated with a similar course of cell-enlargement.

The characteristic varietal size is shown to be determined primarily by the degree of cell-multiplication occurring after pollination. Varietal size was not foreshadowed in the constitution of the flower. Varietal shape was to some extent foreshadowed in the dimensions of those parts of the flower from which the fruit develops.

Seasonal variation in mean fruit-size within a given set of trees of the same variety was associated with variation in both size and number of cells in the flesh. Either or both of the factors might be determinative for fruit-size in any one particular season.

The Bramley's Seedling apple, the only triploid variety studied, was found to occupy an anomalous position in this group of varieties. In the pre-pollination stages the cells were both larger and more numerous and the volume of tissue was greater than that in other varieties. After pollination there was less cell-multiplication and more enlargement when comparison was made with other varieties.

The work described above was carried out as part of the programme of the Food Investigation Organisation of the Department of Scientific and Industrial Research. The author wishes to acknowledge the assistance of Messrs. D. N. Rhodes, C. J. North, and W. W. Freeman.

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On the Nucleolus and Nucleolar-organizing Chromosomes of *Spirogyra*

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With Plates I and II and eight Figures in the Text

INTRODUCTION

THE following is the first part of an account of mitosis in four species of *Spirogyra* in which the points established are: (1) Two nucleolar organizing chromosomes are present. (2) Two nucleoli are organized which may fuse to one. (3) The nucleoli contain coiled structures through which the greatly elongated nucleolar organizing regions of the chromosomes pass. These are designated 'organizer tracks'.

The slow dissolution of the nucleolus has been followed through prophase to metaphase. At mid-prophase a stage is reached when the nucleolus has disintegrated, but when the organizing regions of the chromosomes, contracted to approximately one-third of their length in the resting nucleolus, are still thickly surrounded by material of the organizer track. The shapes and sizes of the nucleolar organizing regions at this mid-prophase stage are somewhat characteristic for each species.

These features are of interest in that nothing has hitherto been known about the relation between chromosomes and nucleoli in *Spirogyra*; this relation, involving the presence of organizer regions of the chromosomes visibly coiled up inside the nucleolus, is unique. A modification of the aceto-carmine stain is presented as the iron-alum aceto-carmine method for algae.

The historical background of *Spirogyra* cytology consists in great part of the assertion by Van Wisselingh (1898 et seq.) that the chromosomes arose from the nucleolus, and its contradiction by Geitler (1930). The observations now made, together with the understanding of nucleolar-organizing chromosomes which has become available since 1931, has made possible a re-interpretation of the older work. The published figures of Moll (1893) and Van Wisselingh (1898 et seq.) of nucleolar structures are confirmed. The work of the above authors is discussed in the appropriate context, and references to the considerable literature will be found in their work and in that of Doraiswami (1946).

MATERIAL

The species of *Spirogyra* investigated have been identified as:

1. *S. crassa* (Kütz.) Czurda emend. 1932. Zygospores were found and the identification is certain.

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2. *S. triformis* Van Wisselingh, 1900. Zygospores were not found. The species, not included in the Susswasserflora (Czurda, 1932), is Van Wisselingh's (1900) and is identified on the basis of the characters of the cell and of the chromosomes.
3. *S. ellipsospora* Transeau, 1914. Zygospores were not found. The species has not been previously investigated cytologically nor recorded in England. Identification is tentative on the characters of the cell.
4. *S. setiformis* (Roth.) Kützing, 1849. Zygospores were not found. The species is identified on the characters of the cell and of the chromosomes and other features of mitosis, as recorded by Geitler (1930 et seq.) and Van Wisselingh (1921). This is regarded as reasonably certain.

A table of vegetative characters (all measurements in μ) is given below.

TABLE I

	Breadth of filament.	Greatest breadth of nucleus.	Diameter of nucleolus when one only is present.	No. of chloroplasts per cell. No. of turns of chloroplast in cell.
S. crassa	150	40–6	14–16	8–10 1 turn
S. ? ellipsospora	120	25	10–12	8–10 1 turn
S. triformis	120	32–5	12	6 1 turn
S. setiformis	105	23–8	10	6 2 turns

Fixation

1. Pre-treatment. A few filaments were held in nitric acid vapour for 1 second.

2. Immediate transfer was made to 1:3 or 1:2 acetic alcohol or to Navashin with the addition of osmic acid. Occasionally (Pl. II, Fig. 1) it was first dipped in $HgCl_2$ saturated solution containing 1 per cent. chromic acid.

Species with thick mucilage sheaths do not fix well.

Staining

Iron-haematoxylin was only occasionally used since the method described below proved more satisfactory. Aceto-haematoxylin gave a good but transitory stain. Aceto-carmine as usually applied was almost useless; with ferric acetate (Thomas, 1940) it was little better.

The iron-alum aceto-carmine method for algae

Fixing times are included here. Any fixative may be used.

1. Fix till all colour is removed from chloroplasts. (*a*) in 1:3 acetic alcohol after nitric pre-treatment, about 5 minutes; (*b*) in acetic alcohol without

pre-treatment, 15 minutes or more; (*c*) in Navashin, 12 hours followed by bleaching, for osmic acid.

2. Water: few minutes.
3. 4 per cent. iron-alum solution in water: 1 or 2 seconds or more.
4. Water: few minutes.
5. Mount in aceto-carmine and heat. Blot if desired. With some species it is better not to blot.
6. Allow slide to remain warm for about 10 minutes.
7. Allow cover-glass to fall off in 95 per cent. alcohol.
8. Mount in euparal or (probably better) transfer for a few minutes to xylol and mount in balsam.

At its quickest, starting from fresh material, this schedule occupies no more than 30 minutes.

A precise and permanent stain results.

The question of artefacts consequent on the use of nitric acid vapour and heating in acetic acid may be discussed briefly. The artefacts produced, if they are to be so described, only make clear the internal organization of the nucleolus; they do not invalidate the observation of nucleolar tracks which are visible in living material examined with the Cooke phase-contrast apparatus. Nitric acid treatment is not always necessary to show them in fixed material. Van Wisselingh (1898) macerated in 50 per cent. chromic acid.

Large vacuoles seen in the nucleolus are due to poor fixation (cf. Geitler, 1930; Doraiswami, 1946) and bubble artefacts are seen in the material of the organizer track (Pl. I, Fig. 1) after over-heating.

The success of the method depends on several things which will be briefly discussed. It is thought that the stain produced is ferric carmine lake, the colour being purple-black or purple-red in the chromosomes, depending on the degree of heating and on the amount of iron present on them after the immersion in iron-alum solution and washing. It has been shown (Seki, 1933) that chromosomes strongly absorb iron from an iron-alum solution, and Gulick (1941) quotes the statement that nucleic acid has an intense affinity for Fe, less than 10^{-9} mg. giving the chromosomes a strong reaction for it. On adding aceto-carmine, the intense bluish-purple colour attributed to ferric carmine lake appears. This is soluble in hot acetic acid; thus the heating process brings about differentiation, more lake being left in the structures which had absorbed most iron. In *Spirogyra* these are the chromosomes including the organizer tracks in the nucleolus and the chromocentres.

Thus, failure of the stain may result from the iron-alum solution being too weak or the time in it too short. Overstaining and lack of differentiation result from too strong a solution of the alum, too long immersion in it, insufficient washing, insufficient heating, dilution of the acid. These things are mentioned because the material may vary somewhat in its requirements and in addition they suggest the ways of improving an overstained preparation.

It may be noted that the success of the method is at variance with the statement of Baker (1945) that the ferric carmine lake is not formed in an acid solution.

For filamentous or thin structures such as are many green algae the schedule given has many advantages; viz. (1) the colour is removed from the chloroplasts without the necessity of bleaching; (2) starch is removed in solution by the heating process. Boiling does not greatly harm the preparations. Mucilage is similarly removed or partly removed.

Thus obstacles to the view of the nucleus are got rid of incidentally to the fixing and staining processes. Other advantages are common to all methods involving heating under a cover-glass, for example, the flattening of the material. This is specially important for filamentous algae, whose usual collapse in xylol is only too familiar, and the avoidance of which has led many workers to rely on long mounting processes in Venetian turpentine or on embedding and sectioning.

RESTING STAGE

The resting stage of the nucleus in all the species of *Spirogyra* that I have examined so far agree in a unique feature, namely, the presence of well-defined structures inside the nucleolus. These are now described as organizer tracks. Van Wisselingh (1898 et seq.) called them *Nukleolusschläuche* or *Nukleolusfaden*. Usually they appear as a deeply staining coil in a paler background, or (Pl. I, Figs. 1, 2, 3) the whole nucleolus appears deeply staining, except for the course of the track, in resting nuclei.

The existence of structures in the nucleoli of *Spirogyra* was observed as long ago as 1893 by Moll and they were more fully described subsequently by Van Wisselingh (1898 and 1921), also of Gröningen. They were overlooked by Geitler (1930 et seq.) and those numerous investigators who have followed his line of interpretation. The resting nucleus of the species of *Spirogyra* referred to in this paper has one large or two smaller nucleoli; two organizer tracks (and presumably organizers) in a large nucleolus, one each in the smaller ones; one or more much smaller spheres of nucleolar appearance—these may be absent; and 'chromocentres' of varying size in a peripheral position in the nucleus.

The *nucleolus* has a firm membrane, well seen on crushing living material. Thus its contents would appear to be physiologically isolated to some extent at least from the rest of the nucleus.

The *nucleolar tracks* are usually not visible in living material unless the nuclei are to some extent crushed and with the ordinary microscope viewed with oblique lighting. The Cooke phase-contrast apparatus, however, shows them up clearly in *S. crassa* at least. With this the interior of the nucleolus is seen to contain loosely coiled threads or thread, brighter than the background, and of width about half that of the corresponding structure seen in a stained preparation.

Pre-treated with nitric acid vapour, fixed and stained, the organizer tracks are more deeply coloured than the background material of the nucleolus (Pl. I, Figs. 1, 2, 3). Often they appear hollow, owing to the material in the middle of the track staining more palely. Whether this is an artefact or not remains to be seen, since it is somewhat characteristic of the species *crassa* and *triformis*, whereas after the same treatment other species not to be fully described here have tracks of equally dense staining properties throughout. In *S.* ? *ellipsospora* (Pl. I, Fig. 3) the tracks are unstained in a heavily stained background; they are shorter and much less coiled than those of *S. crassa*. In *S. setiformis* after the treatment described the two slightly coiled tracks are in a peripheral position in the nucleolus, deeply stained in a pale background, while the centre of the nucleolus is occupied by one or more large globules (Pl. I, Fig. 2).

In contrast to the varied appearance of the track in the nucleolus of the resting nucleus is the much greater constancy of that of the organizing region of the chromosome during prophase (Pl. I, Figs. 4, 5, 6). The presence of two tracks in a nucleolus is best seen in recently divided nuclei while the nucleoli are still being organized. The tracks at first form independent coils; later they become more spread out and difficult to distinguish one from the other.

The small spheres of nucleolar appearance are undoubtedly the *Binnenkörper* and *Nebenkörper* of other authors (Czurda, 1922; Geitler, 1930). Their staining is like that of the background material of the nucleolus, and they are usually situated near this body in the cell. Not more than two per cell have previously been recorded, but three have occasionally been observed in my preparations. Probably there is no significance in the number of these bodies, and a reference to the development of nucleoli in telophase (to be described in a later paper) makes it likely that they are merely globules of nucleolar material which have failed to become organized. They do not become broken down during prophase as the organized nucleolus does and may still be seen at metaphase apparently unchanged. Thus it appears probable that in *Spirogyra* the nucleolar organizer is also, or is connected with, the agent causing nucleolar disorganization in prophase. These globules are not of constant occurrence in my material of *S. crassa*, *S. triformis*, and *S.* ? *ellipsospora*.

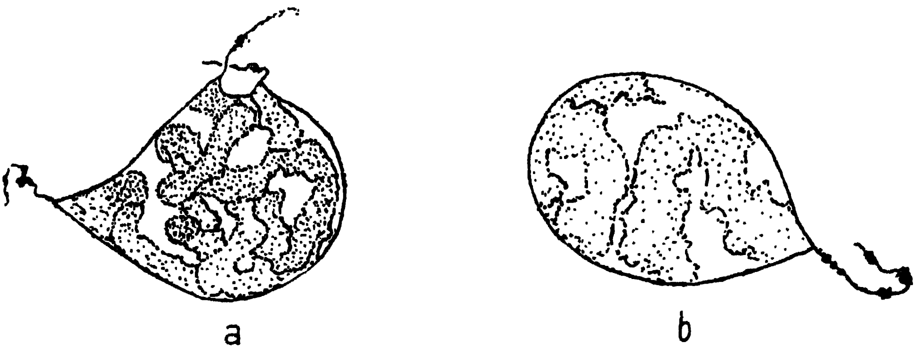
The *chromocentres*, so called following Geitler (1930), are seen as deeply stained bodies around and just within the periphery of the nucleus. In view of the difficulty of fixing chromosome structures in the resting nucleus, little significance can be attributed to their shape, which is in *S. crassa* and *S. triformis* irregular, often more or less linear, in *S. setiformis* globular. In *S.* ? *ellipsospora* they come into the category of dots and granules. Similar dots and granules are present in the other species together with the larger bodies in every gradation of size. Two points may be of interest: (*a*) their position is always peripheral; (*b*) species with large/small chromocentres have large/small chromosomes.

Geitler considered that *S. setiformis* had no chromocentres. On the other hand, he and Suematsu have stated that in certain species the number of chromosomes is the same as the number of chromocentres, and according to

Geitler the position of the chromocentres corresponds in two daughter-nuclei (Geitler, 1935*b*). It has not been possible to confirm these observations in the present investigation, and in particular *S. triformis* has approximately at least twice as many large chromocentres as chromosomes.

It may be of interest that in old filaments, whose cells will probably not divide again, of *S. crassa* and *S. triformis* the chromocentres are larger and more deeply staining than normal and the nucleoli smaller and less deeply staining.

Geitler (1935*b*), Yamaha (1935), and Suematsu (1936) have obtained a positive reaction with Feulgen's stain in the chromocentres of various species of *Spirogyra*. It may be doubted whether these so-called chromocentres are altogether comparable with those of higher plants. They presumably indicate the presence of a good deal of heterochromatin in the chromosomes of several species.



TEXT-FIG. 1. *S. crassa.* Two nucleoli from early prophase nuclei. Parts of the nucleolar-organizing chromosomes in prophase state attached at the ends of the organizer tracks in the nucleolus. The nucleolar membrane is pulled out into a point at the spot where the chromonema emerges from it.

The *chromosomes* are represented in the resting nucleus not only by the 'chromocentres'. At least in *S. crassa* and *S. triformis* after fixation, what are apparently fine closely and irregularly coiled chromonemata fill the interior of the nucleus. It might therefore be regarded as a solid nucleus in Manton's sense, although it is somewhat different from that of *Allium* (Manton, 1935). In these two species there is evidently a pronounced barrier between the nucleoli, since two nucleoli are quite commonly found, although fusion may take place during telophase, when presumably the chromosome are not yet fully expanded. These species have large chromosomes. By contrast the species ? *ellipsospora* and *setiformis* with small chromosomes practically always have but a single nucleolus in the mature nucleus, although two are always initiated at telophase. In them it is conceivable that the chromosomes are smaller or more movable structures at resting stage.

It is clear that a fine chromonema or structure representing a chromonema must extend throughout the organizer track; this chromonema is the nucleolar organizer. A theoretical diagram of the resting nucleus may therefore be constructed (Text-fig. 6, *a*) applicable to the species named in this paper, and based specially on *S. crassa.*

PROPHASE

The prophase is characterized by the long-drawn-out breakdown of the nucleolus, and the gradual emergence in a striking form of the nucleolar-organizing regions of the two nucleolar-organizing (N.O.) chromosomes (Pl. I, Figs. 4, 5, 6). The structure of the prophase chromosomes themselves will not be considered at this time.

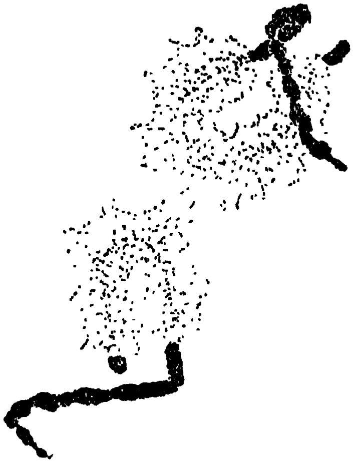


FIG. 2

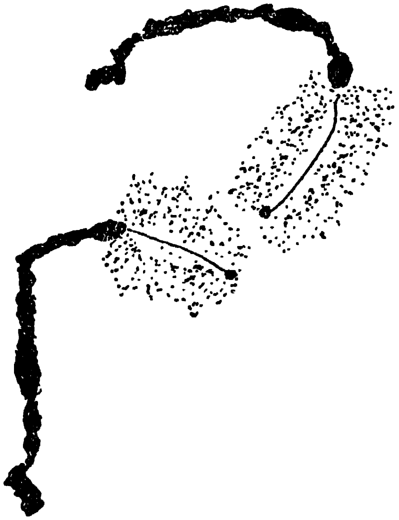


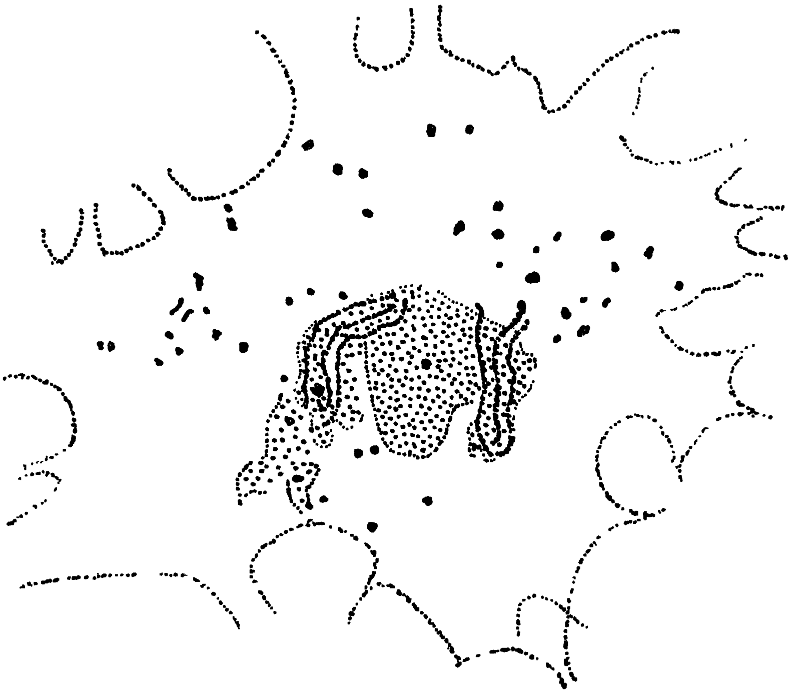
FIG. 3

TEXT-FIG. 2. *S. crassa.* Mid-prophase; two nucleolar-organizing chromosomes like Pl. I, Fig. 4. The N.O. regions are horseshoe-shaped and sub-terminal, the 'satellite' large.

TEXT-FIG. 3. *S. triformis.* Mid-prophase, the two nucleolar-organizing chromosomes on Pl. I, Fig. 5; Pl. II, Figs. 1 and 2. The N.O. regions are sub-terminal, the 'satellite' very small. The regions are straight.

In *S. crassa* and *S. triformis* there is first of all an increase in size of the nucleolus as a whole, together with a contraction in length but increase in width of the organizer tracks. They gradually uncoil and eventually assume a horseshoe shape, the two being clearly distinct from one another when both are included in the same nucleolus. The material composing the track becomes more diffuse in appearance and does not stain so deeply. At this stage it is often possible to see the connexion between the end of the track and the attached portion of chromosome, the nucleolus being pulled out into a small projection surface at the point of connexion (Text-figs. 1, 7). This pulling out is probably caused during the process of flattening the thread under the cover-glass. The maximum number of such connexions to the parts of the

N.O. chromosomes that should be observable is four. Three have actually been observed in one nucleolus—reasonable confirmation of the positions of the chromonemata in relation to the organizer tracks shown in Text-fig. 6, *a*. Van Wisselingh observed the attachment of the long arms of the chromosomes to the nucleolus (Text-fig. 7), but not that of the short distal region or satellite. As prophase proceeds the nucleolus loses its firm outline and the material



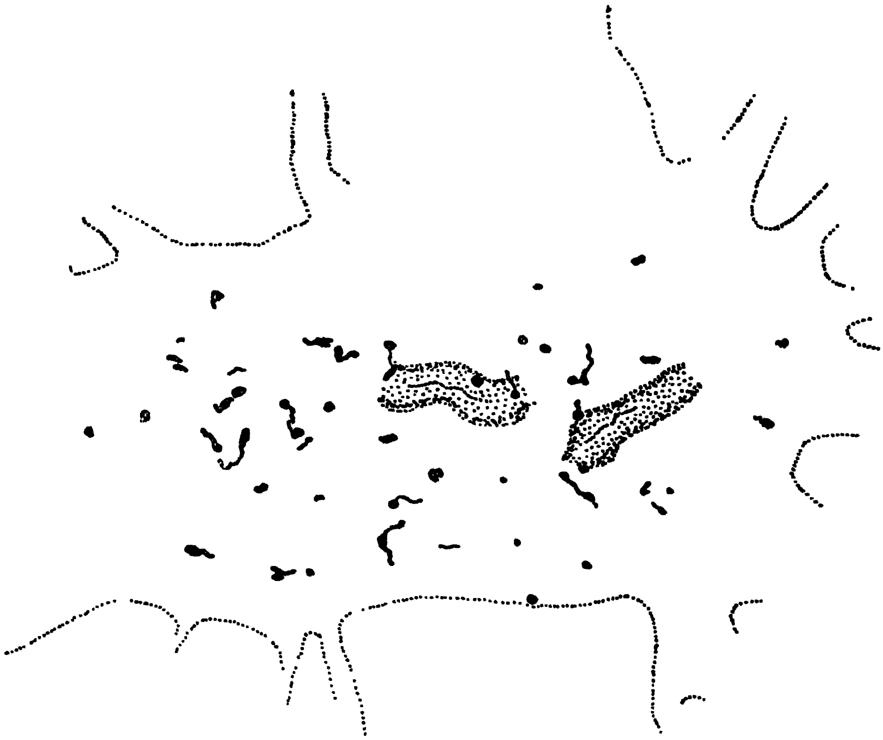
TEXT-FIG. 4. *S. ? ellipsospora*. Prophase, slightly earlier than mid-prophase. The two nucleolar-organizing regions are partly freed from the mass of dispersing nucleolar material and the nucleolar membrane has disappeared. Each organizer in the form of a chromonema lies in an apparent tube of deeply stained material, the organizer track material. Other chromosomes are minute.

composing it slowly disperses. Finally the organizers with the track material round them are separated and free in the nucleus; they are further contracted and the dispersal of the track material is also going on. They are now obviously part of the N.O. chromosomes (Pl. I, Figs. 4, 5, 6).

The organizer at this stage appears as a fine chromonema, palely staining, subterminal in position. The terminal portion of chromosome is small in *S. triformis*, larger in *S. crassa*. Around the organizer lies the track material, at this stage striated by lines radiating from the organizer, and having a woolly outline. It has the appearance often associated with heterochromatin. Thus *mid-prophase* has been reached, a stage characterized in *Spirogyra* by the presence of the two organizers in a partly expanded state.

The gradual way in which this stage has been reached during the breakdown of the nucleolus is the proof of the structure of the nucleolus in the resting nucleus, and its relation with the N.O. chromosomes, shown in Text-fig. 6, *a*.

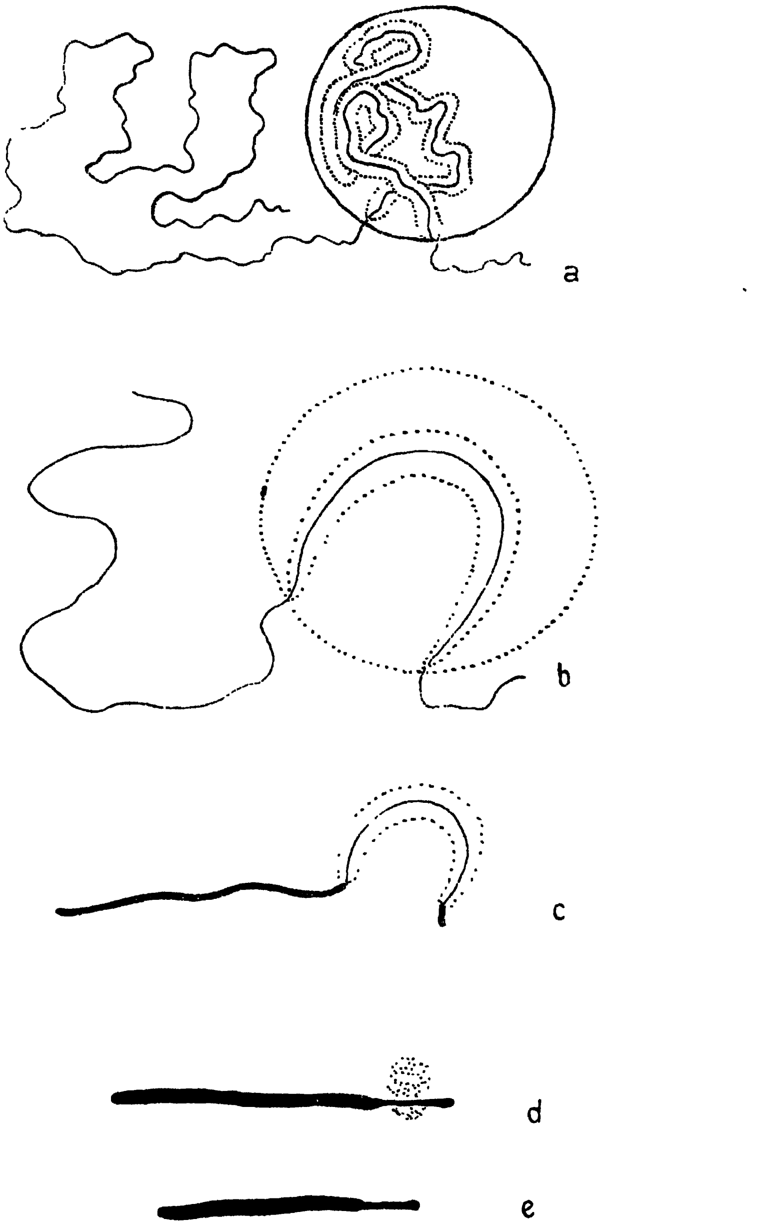
The mid-prophase of *S. triformis* is shown in Pl. I, Fig. 5, and Pl. II, Figs. 1 and 2, the same preparation in three different degrees of magnification. Pl. II, Fig. 2, shows the two N.O. chromosomes with the organizer regions; the width of the chromonema traversing the track material is somewhat exaggerated in the photograph owing to the limitations of the optical system (cf. Text-fig. 3). The small satellite is scarcely visible.



TEXT-FIG. 5. *S. ? ellipsospora*. Mid-prophase, shown in Pl. I, Fig. 6. Two nucleolar-organizing or N.O. chromosomes, consisting entirely of nucleolar-organizing region. The regions are more or less straight. Other chromosomes are minute.

In *S. crassa* at the same stage the organizers are somewhat longer and still curved (Pl. I, Fig. 4). The long arms of the N.O. chromosomes were out of focus in this photograph (cf. Text-fig. 2), but the larger 'satellites' are visible, showing the subterminal position of the organizer.

Van Wisselingh, who alone among the many investigators of *Spirogyra* found the mid-prophase stages described above (cf. Pl. I, Fig. 4 with his figure, here reproduced as Text-fig. 7), was influenced by the theory current in his time of the origin of chromosomes by fragmentation of a single 'spireme' thread.



TEXT-FIG. 6. Diagrammatic representation of nucleolar-organizing or N.O. chromosome such as that of *S. crassa*. (*a*) At resting stage, the N.O. region coiled up inside the nucleolus; the region consists of a fine chromonema, embedded in a thick coating of deeply staining material, the organizer track. (*b*) At early prophase, the N.O. region contracted and no longer coiled, the organizer track broadened and the nucleolus as a whole expanded. (*c*) At mid-prophase, further contraction of all parts of the chromosome; the nucleolus has become dispersed; the N.O. region has a characteristic shape and appearance. The chromonema passing through the track material is thick enough to stain distinctly at this stage. (*d*) At later prophase, nearly maximum contraction; the N.O. region is now much thickened and the last remains of the track material lie round it loosely. (*e*) At metaphase; no sign of the nucleolus remains. All to the same scale except the satellite in (*a*) and (*b*) and the long chromosome arm in (*a*) which are arbitrarily represented. (See Table II, p. 50.)

Observing the attachment of the long chromosome arm to the organizer track, he concluded that the chromosome had emerged from the nucleolus. Therefore all the chromosomes must originate there.

Geitler and many others had no difficulty in demolishing this interpretation by establishing that the chromosomes originated outside the nucleolus. No attempt was made to find the 'nucleolar tubules'. Yet it may be seen from Geitler's figures of the nucleolus in resting stage of *Spirogyra X* (Geitler, 1935*a*) and mid-prophase of *S. crassa* (Geitler, 1930) that he may have had some evidence before him.

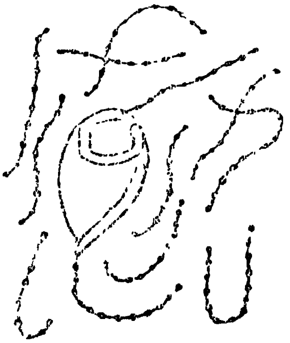


FIG. 7

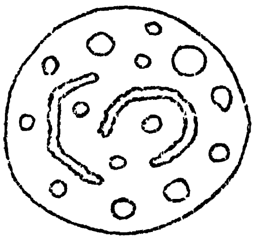


FIG. 8

TEXT-FIG. 7. *S. crassa*. Mid-prophase. (After Van Wisselingh.)

TEXT-FIG. 8. *S. setiformis*. Mid-prophase. (After Van Wisselingh, 1921.) Two curved nucleolar-organizing chromosomes consisting entirely of organizer regions, and fragments of material derived from the breakdown of the nucleolus.

Van Wisselingh's figure of the mid-prophase of *S. setiformis* is also (Text-fig. 8) reproduced; the same stage was found once in the present investigation. It shows two rather long organizers with track material and a number of lumps of other material which are found scattered about in the nucleus after the disorganization of the nucleolus.

In this species, as in the following one, no other chromosome arms or parts appear attached to the organizer, which is itself a complete chromosome and the largest of the complement. This state of affairs does not appear to have been recorded for any other organism.

In *S.* ? *ellipsospora* the breakdown of the nucleolus proceeds along lines similar to those followed in the previously described species. The organizers being less coiled from the first are practically straight, with a large mass of dispersing nucleolar material between them at an early stage (Text-fig. 4). There can be no doubts in this species as to the 'hollowness' of the organizer track (Text-fig. 4). It is clearly differentiated into a central paler staining region and a thin deeply staining outer region, the organizer itself in the form of a thin thread running through the centre of the pale staining region. It thus has the appearance of lying in a hollow tube of deeper-staining material.

At mid-prophase, the remainder of the nucleolar material having dispersed, the two organizers lie, surrounded by their track material, free in the nucleus, as in the other species (Pl. I, Fig. 6, and Text-figs. 4 and 5).

It is possible to show (see Pl. I, Figs. 4, 5, 6, all to the same scale) that the organizing regions of the N.O. chromosomes of the three species, all relatively large ones, are of the same order of size. Taking into account Van Wisselingh's figure of *S. setiformis* (Text-fig. 8), we have here four species each characterized by the shape of the organizer and other features of the N.O. chromosome at mid-prophase.

Late prophase stages show continued contraction of the organizer and dispersal of the track material. In *S. crassa* (Text-fig. 6, *d*) the last of it forms a loosely associated patch of granular material at a time when the chromosome is almost fully contracted and the organizing region itself has somewhat thickened.

In all the species the track material, and quite definitely the whole of the organized nucleolus, has dispersed when metaphase is reached.

General summary of the changes in the N.O. chromosome from resting stage to metaphase

Text-fig. 6, *a*, *b*, *c*, *d*, shows these changes diagrammatically: it is based in a general way on *S. crassa*. All the structures shown are on the same scale except the parts of the chromosome outside the nucleolus at resting stage and early prophase (*a* and *b*) which are arbitrarily represented. Only one of the two N.O. chromosomes is shown. No attempt has been made to show the structures of the chromosomes which will be described later.

The table below gives the dimensions in μ for *S. triformis*.

TABLE II

Dimensions of Nuclear Structures (in μ)

	Diameter of nucleus.	Diameter of nucleolus and organizer track.	Total length of N.O. chromosome.	Length of N.O. region.	Length of long chromosomes.	Length of short chromosomes.
Resting nucleus	30	12, 2·0	—	25	—	—
Early prophase	30–60	17, 3·5	41	15	26	—
Mid-prophase	—	—	25–7	8	17–18	15
Metaphase	—	—	10–12	2	8–9	7

Both diagram and table show the initial expansion of nucleolus and organizer track in early prophase. This and the striation of track material in mid-prophase are both evidence of the active chemical changes taking place. The elongation of the organizer to a length of 25 μ in the resting nucleolus is striking; since there are two such organizers, a single nucleolus may thus contain a length of some 50 μ of N.O. chromosome.

THE NATURE OF THE ORGANIZER TRACK

It appeared possible to consider this a specialized part of the nucleolus, formed in proximity to the organizer, whose differential staining might be due to the fact that it is composed of substances forming a step in the synthesis of the materials composing the rest of the nucleolus. When the track appeared 'hollow' there might be two such steps, the substance next to the organizer staining little, the next layer deeply, and material outside this less deeply. On this view, the substance round the organizer (itself an independent structure having the form of a chromonema) was nucleolar material if only partly synthesized, and the name 'nucleolar tubule', a translation of Van Wisselingh's designation, was thought appropriate (Godward, 1947). I am indebted to Dr. D. G. Catcheside for the following alternative view of it: the tubule may be some special substance on the outside of the permanent part of the organizer and separating it from the nucleolar substance. In this case there would be an interface between the organizer outer substance and the nucleolar substance, at which stain behaving specially might give the appearance of a hollow tube. The contraction of the 'nucleolar tubules' goes *pari passu* with that of the satellite stalk or N.O. region. Hence they are effectively parts of the same structure; the 'nucleolar tubule' is organized by, or is part of, the nucleolar organizer rather than part of the nucleolus. There is evidence for a duality of the structure of the organizer so long as the nucleolus is present and for a short time after it vanishes. Further species of *Spirogyra* have now been found in which the organizer track is definitely not hollow and in one species has a delimiting layer of apparently membranous nature. The second interpretation therefore is preferable, and the term 'nucleolar tubule' has been abandoned in favour of the relatively non-committal name 'organizer track'.

METAPHASE

A few comments only on metaphase are given here. The metaphase chromosomes of the four species described are as follows: *S. triformis*, 6 large; *S. crassa*, 12 large; *S. ? ellipsospora*, ? 70 minute; *S. setiformis*, unstainable (not counted).

The six chromosomes of *S. triformis* comprise two N.O. chromosomes, two other long chromosomes, and two short chromosomes. Since this is the haploid complement, there appears to be evidence of polyploidy even in this species. It is a curious fact too that all the species described in this paper organize two nucleoli although, as in the species *setiformis*, they may nearly always fuse in early telophase. Among other species examined two only have been found in *Spirogyra*; in other genera of Conjugales as many as four nucleoli of different sizes have been found in *Sirogonium*, two occasionally, but more often one, in *Cosmarium*, so far only one in *Zygnema*.

HETEROCHROMATIN

Since the N.O. regions of the chromosomes are paler staining than the rest of the chromosomes at metaphase, they may be regarded as heterochromatic. It must be pointed out, however, that this result has not been obtained with Feulgen's stain, which so far has not worked with these species, in the hands of the writer. If the N.O. regions are heteropycnotic at metaphase they might be expected to be so at resting stage, where they are represented by a fine chromonema surrounded by the organizer track. The chromonema itself is certainly not stained at resting stage; its existence has to be deduced from early prophase stages, and it is in the same state as the other chromosomes. There remains the organizer-track material, which is certainly deeply stained with carmine but generally no more so than the rest of the nucleolus unless there has been pre-treatment with nitric acid vapour. Several investigators have used Feulgen's stain on various species of *Spirogyra* and obtained a positive reaction in the chromosomes. Not one of these authors (Geitler, 1935*b*; Suematsu, 1936; Doraiswami, 1946) obtained any positive reaction in the nucleolus, nor did they see any organizer tracks. It therefore seems hardly correct to regard the N.O. regions as heteropycnotic at resting stage. Positive heteropycnosis is shown at that stage by the 'chromocentres' when Feulgen's stain is used (Geitler, 1935*b*).

SUMMARY

1. The role of nucleoli in the early stages of mitosis has been followed in four species of *Spirogyra*.

2. Two nucleolar organizing (N.O.) chromosomes are present.

3. The nucleoli contain coiled structures through which greatly elongated nucleolar organizing regions of the chromosomes pass. These are designated 'organizer tracks'.

4. An iron aceto-carmine method for staining algal material is described.

CONCLUDING REMARKS AND ACKNOWLEDGEMENTS

Numerous species of *Spirogyra* have been examined in less detail than the four described. All appear to have essentially the same kind of nucleolar organization, although there appear to be considerable differences of detail. The same is true of *Zygnema* and *Closterium*. It may be too soon to assume, but it seems possible that nucleolar tracks will be found in the nucleoli of all Conjugales.

The author is especially indebted to Dr. D. G. Catcheside and Prof. I. Manton for confirmation of the observations and discussion of the manuscript; to Prof. Manton for Fig. 1, Pl. II, and also to the Central Research Fund of the University of London for the grant of £180 with which the Cooke microscope and accessories used in the investigation were purchased.

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EXPLANATION OF PLATES

Illustrating M. B. E. Godward's article, 'On the Nucleolus and Nucleolar-organizing Chromosomes of *Spirogyra*'.

PLATE I

FIGS. 1, 2, 3. Nucleoli of three species of *Spirogyra* showing organizer tracks.

FIG. 1. *S. crassa* (× 2,250). Bubble artefacts in track.

FIG. 2. *S. setiformis* (× 2,330). Organizer tracks in peripheral position within nucleolar membrane; centre of nucleolus occupied by a globule.

FIG. 3. *S. ? ellipsospora* (× 2,250). Organizer tracks unstained, probably corresponding to central unstained part of organizer tracks of the other species.

FIG. 4. *S. crassa* (× 1,040). Mid-prophase, the two horseshoe-shaped bodies are the nucleolar-organizing (N.O.) regions of the N.O. chromosomes. The attached long arms of these chromosomes are not in focus. The small distal regions or 'satellites' are seen one at the upper left-hand end of the region on the left, one at the lower right-hand end of the region on the right. Parts of the other 10 chromosomes are also visible. (Cf. Text-fig. 2.) (× 1,250.)

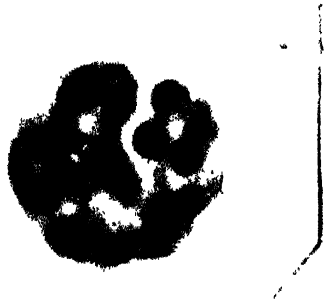
FIG. 5. *S. triformis* (× 1,040). Mid-prophase. Six chromosomes, two N.O. chromosomes, each with a straight nucleolar organizing region, to one end of which a long chromosome arm is seen attached. (Cf. Text-fig. 3.)

FIG. 6. *S. ? ellipsospora* (× 1,040). Mid-prophase. Two N.O. chromosomes consisting entirely of nucleolar-organizing region: traces of the other minute chromosomes are seen with difficulty. (Cf. Text-fig. 5.)

PLATE II. *Spirogyra triformis*

Fig. 1 (× 225), showing the cell with mid-prophase seen in Pl. I, Fig. 5.

Fig. 2. The nucleolar-organizing chromosomes of the cell shown above (× 2,900). The chromonema-like organizing region of the chromosomes passes through a dense mass of deeply staining material (organizer-track material). The position of the organizing region is subterminal, although the 'satellite' is very small. (Cf. Text-fig. 5.)



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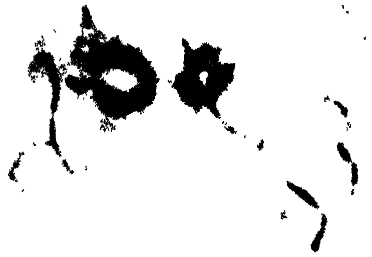

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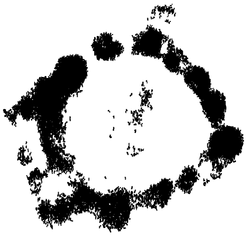

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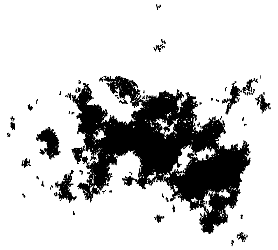

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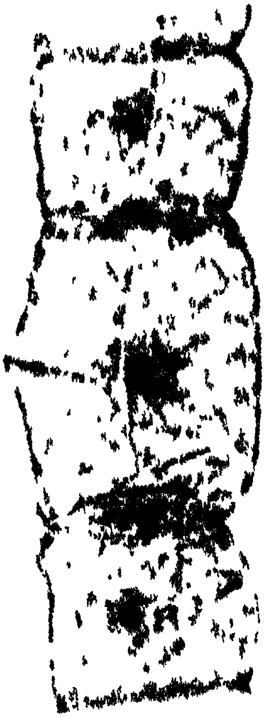
M. B. E. GODWARD—*Spirogyra*



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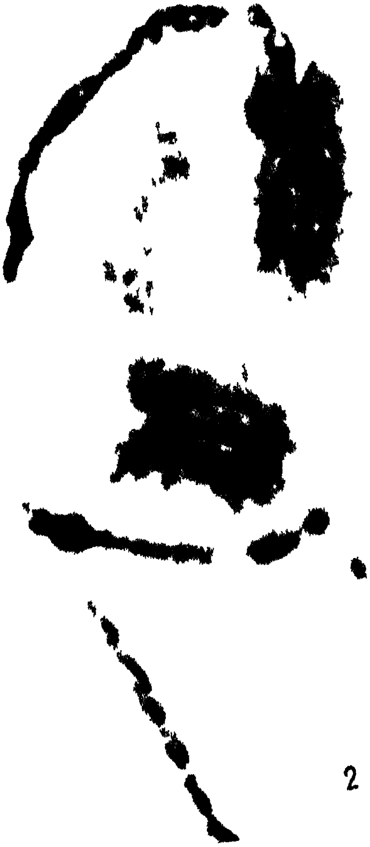

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M. B. E. GODWARD—*Spirogyra*

Physiological Studies in Plant Nutrition

XV. Assimilation of Carbon by the Ear of Barley and its Relation to the Accumulation of Dry Matter in the Grain

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With four Figures in the Text

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INTRODUCTION

THE dry weight of the ear of barley constitutes at harvest about 50 per cent. of the total dry weight of the aerial parts, and this large fraction of the dry weight is accumulated in the main over a period of 4 to 5 weeks as compared with about 10 weeks required for the growth of the vegetative organs. It is apparent, therefore, that either there must be a considerable transfer to the ear of carbohydrate synthesized during the vegetative stage of growth, as postulated in the well-known migration hypothesis, or very active assimilation must accompany the growth of the ear. In a long series of experiments dealing with sugar metabolism in barley (Archbold and Mukerjee, 1942; Archbold and Datta, 1944; Archbold, 1945) no evidence was obtained of migration on a scale which would be effective and it was concluded that the carbon needed for starch synthesis in the grain was supplied by current assimilation. During the time of rapid grain development the number of functional green leaves is rapidly diminishing, and it was long since pointed out (Déhèrain and Dupont, 1901) that the assimilatory capacity of the leaves at this time was unlikely to be adequate to supply the material for grain development. It is therefore necessary to consider the functional importance of the other green surfaces as assimilating organs and in particular of the ear itself. By shading ears of wheat (Boonstra, 1929) or barley (Watson

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and Norman, 1939; Archbold, 1942) it has been found that the dry weight of the ear is reduced by 20 to 30 per cent., and there is also evidence of the functional importance of the flag-leaf sheath (Archbold, 1942), and in oats stems have been shown to have assimilatory capacity (Stålfelt, 1935). Alternative estimates of the assimilating power of the ear were made in the experiments of Boonstra by leaving only the ears exposed to light and in those of Archbold by estimating the contribution of the ear in the absence of leaves. The values obtained varied somewhat widely from those found by simply shading the ear, indicating important effects on metabolism other than prevention of assimilation by the shaded organs. In order to obtain a valid estimate of the contribution made to its own dry weight by assimilation in the ear it is therefore necessary to measure directly the carbon dioxide uptake. Accordingly a continuous record has now been made of carbon dioxide uptake by day and emission by night by the barley ear under natural outdoor conditions, from the time of emergence until assimilation ceased with loss of all the chlorophyll. The results reported here appear to confirm the values found when ears only were shaded, rather than those found when leaves were removed in addition to ear shading, or when ears only were illuminated, in the earlier experiments. At the same time dry-weight increases and starch content of the ears has been determined for comparison with the amount of carbon dioxide assimilated.

METHODS AND MATERIALS

Apparatus for measuring assimilation and respiration of barley ears

A diagram of the apparatus used for measuring carbon dioxide uptake by the ears is shown in Fig. 1. The design was adapted from that described by Chinoy (1935) and due to Professor F. G. Gregory, for measuring assimilation in leaves under outdoor conditions. The amount of carbon dioxide assimilated is measured as the difference between that absorbed from normal air and that absorbed from air which has passed over the surface whose assimilating capacity is to be determined. The essential features of the apparatus are that the gas stream used is sufficiently rapid to prevent marked depletion of the normal CO_2 of the air, while at the same time ensuring complete absorption of CO_2 after passage through the assimilation chambers, and that the gas streams in replicate circuits flow at equal rates.

Ears, attached to the plants, are enclosed in double-walled glass chambers (A), 30 cm. long and internal diameter 3·5 cm. The lower end of the chamber is left open to the air and to the upper end is sealed a glass tube (B) leading to the absorption vessel (C). Water can be circulated in the outer jacket of the chamber if desired, for cooling purposes. The absorption vessel consists of a tube 75 cm. long and diameter 2·5 cm., fitted by means of a rubber stopper to a wider tube provided below with a bulb, volume 200 ml., bearing a side arm. Below the bulb is a fine tubulure closed by a glass tap. The long tube rests upon the base of the bulb and is filled with solid glass beads to a height of 60 cm. N/1 NaOH is introduced into the bulb, and when air is flowing the liquid is raised to within 3 or 4 cm. of the top layer of beads, and the gas stream broken up into small bubbles. In this way complete absorption of CO_2 from normal air is obtained with flow rates up to 50 litres an hour. Baryta traps (N) are attached to the absorption tower, so that any failure to remove all the CO_2 may be readily detected. The tower is connected by means of a three-way tap (D) and a small needle valve (E) to a rotatory pump at (F) by means of which air is drawn through the apparatus. Six absorption towers are used in parallel connected to the pump by a common lead at (G). This lead carries a branch open to the air through a large needle valve (H). This valve is used to control the proportion of the total inflow which passes through the apparatus. Only a small part of the inflow to the pump is required to maintain appropriate flow rates in the towers, which can thus be readily adjusted.

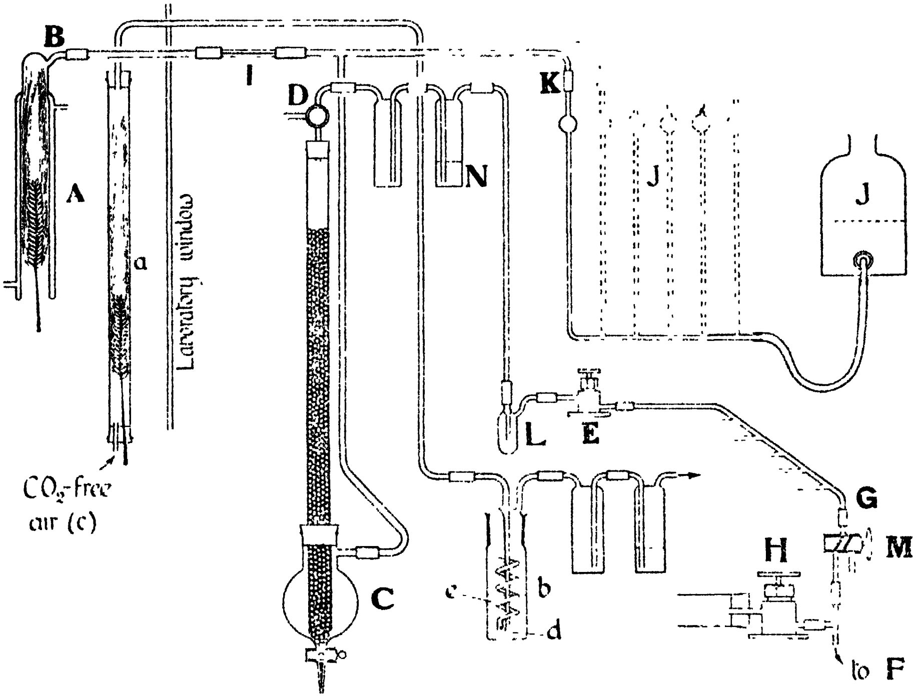

Fig. 1. Apparatus for determining assimilation and respiration by barley ears attached to the plant.

To maintain equal flow rates in the six circuits, capillary tubes about 10 cm. long and of equal resistance to flow are introduced between the assimilation chambers and the absorption towers at (I), and the pressure drop between the atmosphere and the inner end of these tubes recorded on a multiple water manometer (J). At the tops of the six manometer tubes small pieces of thermometer tubing are inserted in the rubber connexions (K) to damp out excessive oscillations. Before use, the capillary tubes are calibrated using the principle of the Wheatstone bridge, as shown in Fig. 2. Three tubes of nearly equal resistance to gas flow are placed in the arms of a bridge composed of glass tubing, at *r*1, *r*2, and *r*3. In the fourth arm is placed the tube to be tested *r*4. Across the bridge is a water manometer, and when the height of the liquid in the two manometer arms is the same, then $r1/r2 = r3/r4$ and is independent of flow rate. A number of tubes of nearly equal resistance to flow is first selected and by trial, placing each in turn in the fourth arm of the bridge, the tubes are brought to the desired value either by grinding the ends or by slightly reducing the end aperture by heating. If this latter procedure is necessary the tubes must always be used the same way round. After standardizing the capillary tubes the apparatus is assembled and the rates of flow in each circuit tested by timing the outflow from a 20-litre aspirator connected to each in turn. At this stage manometer heights corresponding to suitable flow rates are marked on squared paper fastened behind the tubes (J). At the start of an experiment the level of liquid in the manometers is adjusted as nearly as possible to the required position by first turning on the pump with the large needle valve (H) fully open to the air, and then slowly closing it until sufficient air flows through the apparatus. Final adjustments to the separate circuits are then made with the small needle valves. It is necessary to incorporate glass bulbs (L) in the leads from the absorption towers and below the level of these valves to prevent condensed water trickling into them. The common outlet tube to the pump was also provided with a tap (M) to remove water if necessary.

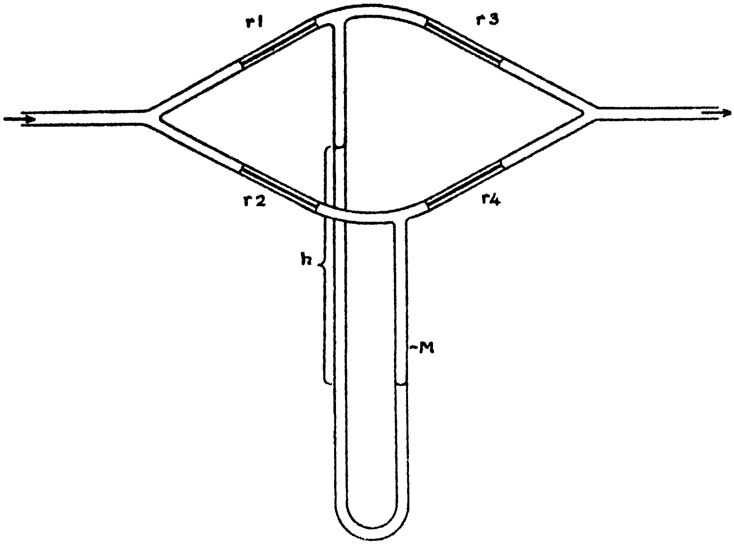

FIG. 2. Apparatus for testing resistance to air-flow of glass capillary tubes.

To conduct an experiment the bulbs and glass beads of the absorption units are first carefully cleaned and dried. The absorber is then assembled together with the three-way tap, and the side arm closed by attaching a small piece of glass rod inserted in a short length of pressure tubing. The unit is now weighed and 50 ml. of N/1 NaOH run quickly in from a burette connected to a reservoir of solution and protected from the carbon dioxide of the air. With the taps turned to exclude air the unit is again weighed and then placed in position for the experiment. When the assimilation period is completed, the three-way tap is turned off, and the side arm of the tower closed with the same glass rod and tubing, and the unit weighed to allow for any loss or gain of water during the experiment. The soda is now run out from the tower by opening the lower tap and allowing air to enter through a soda-lime tube attached to the three-way tap. It is collected in a 250-ml. flask containing 100 ml. of 10 per cent. $BaCl_2$, which precipitates the carbonate and at the same time rapidly dilutes the excess NaOH. Finally the unit is weighed once more so that the amount of NaOH run out may be determined. By this weighing technique the necessity for quantitative removal of the NaOH from the tower is avoided. After standing to allow carbonate to settle, 50 ml. of the supernatant liquid are measured out and a known volume of N/10 HCl, insufficient to neutralize all the excess NaOH, added by means of an automatic pipette. The remaining NaOH is then titrated with a further quantity of N/10 HCl and the amount of carbonate present found by subtracting the total titration value from that of an appropriate blank. A specimen calculation is shown below:

	Weight of absorption unit g.	Wt. NaOH g.
1. Empty	611·20	—
2. + 50 ml. N/1 NaOH, before experiment . . .	663·35	52·15
3. + „ „ after „ . . .	662·25	51·45
4. After running out NaOH	617·95	44·70

44·70 g. NaOH made up to 250 ml. and aliquots titrated with HCl.

$$\text{ml. N/10 HCl required for 50 ml. NaOH} \begin{matrix} (1) & 26{\cdot}43 + 18{\cdot}30 \\ (2) & 26{\cdot}43 + 18{\cdot}25 \end{matrix} \Big\} \text{ Total } 44{\cdot}71.$$

Hence ml. N/10 HCl required for excess NaOH in 52·15 g. taken =

44·71 × 5 × [51·45/44·70] = 257·31
ml. HCl required for blank = 469·91
Hence ml. HCl equivalent to CO_2 absorbed = 212·60

Duration of experiment 42 hours, hence mg. of CO_2 absorbed in 42 hours = 467·7 or 11·13 per hour.

After running out the NaOH the absorption units are dismantled and the beads and glass parts washed free from alkali and dried ready for reassembling.

Details of the respiration apparatus are included in Fig. 1. Ears attached to the plant are enclosed in glass tubes (*a*), diameter 2·5 cm., closed at the top with a one-holed rubber stopper, carrying a glass tube leading to the absorption bottles (*b*), and at the bottom with a two-holed stopper. This stopper is slit up the side to allow the peduncle of the ear to be introduced into one of the holes, and the joint made air-tight with a mixture of bees-wax and vaseline.

Air freed from CO_2 by passage through 30 per cent. KOH and a 2-ft. soda-lime tower at (*c*) is drawn through the glass tube inserted in the second hole of the bottom stopper, and enters the absorption bottle through a fine jet (*d*) over which rests a glass spiral (*e*). This device breaks up the gas stream and so ensures complete absorption. The CO_2 is determined in the same manner as described for the measurement of assimilation, by weighing the absorption bottles containing the NaOH, here N/2, and suitable titration of the excess remaining at the end of the experiment.

Material

Barley, Spratt Archer, was grown in the open in 10-in. glazed pots in the summer of 1946. Eight seeds per pot were sown on May 2 and complete mineral nutrients given in three equal doses on May 14, when the seeds had germinated, on May 28 as the third leaf emerged, and on June 11 as the fifth leaf emerged. The total amounts of salts added per pot were: $Na_2HPO_4.12H_2O$, 2·52 g.; K_2SO_4, 1·85 g.; $NaNO_3$, 9·0 g.; $MgSO_4.7H_2O$, 1·25 g.; $CaCl_2.6H_2O$, 0·37 g.; $FeCl_3$, 0·15 g.; $MnSO_4.4H_2O$, 0·10 g. At the time of the second dose the plants were thinned to four per pot, and at the fifth-leaf stage the main axis on each plant marked. Only ears on these main axes were used in subsequent experiments.

Assimilation measurements were made during the day and respiration measurements during the night on ears attached to the plants and subject to the natural fluctuations of light and temperature. The measurements were begun at the time of ear emergence. Three pots were set up outside the laboratory window, and an ear on the main axis of one plant from each pot introduced into an assimilation chamber; three empty chambers, to act as controls, were placed over the pots at the same height as those containing ears, and all six chambers connected up to the absorption units inside the laboratory. Air was then drawn through the chambers at 25 litres an hour, as already described, during the daylight hours of three successive days. At dusk the taps were closed to exclude air, the gas stream turned off, and the chambers lifted off the ears, and at dawn the chambers were replaced and the gas stream again started. A second set of ears from the same pots was introduced into the respiration chambers at dusk on the first day and the carbon-dioxide-free gas stream allowed to flow during the dark hours of three successive nights, at about 6 litres an hour. At the end of the 3-day period the ears were finally removed from the chambers, detached from the plants, weighed, and preserved in alcohol for dry weight and starch determinations. The assimilation and respiration absorbers were removed for estimation of the carbon dioxide absorbed, and were at once replaced by a second set. Another group of three pots was set up as quickly as possible and connected to the second set of absorbers and the gas stream started. The time taken to change the plants and absorbers was from 10 to 30 minutes. This procedure was repeated until assimilation ceased, with loss of all the chlorophyll, as the ears reached the full ripe condition. Eleven sets of observations were made, covering a period of 4½ weeks, during which ears were assimilating, together with two sets of measurements after assimilation had ceased, and three sets on the flag leaf and its sheath before ear emergence.

Dry weights were separately determined on the whole ear and on the grain, giving, by difference, the dry weight of the awns, rachis, &c. For the estimation of starch, ears preserved in alcohol were transferred to Soxhlet thimbles, and extracted with fresh alcohol overnight. The material was then dried and weighed, and the grain separated and weighed. The grain was then finely ground in a Wiley mill, and aliquots of the powder used for estimation of starch by the method of Hanes (1936). The same β-amylase preparation was used for all the determinations, and the hydrolysis limit found for a preparation of starch made from a sample of the barley used for sowing the experimental pots. Under the conditions used, namely, 24 hours' incubation at 26° C. and pH 4·8, the percentage hydrolysis was 59·5, the mean of six determinations carried out along with the estimations of starch in the experimental samples. The reducing power of the hydrolysates was determined using Somogyi's (1945) reagent recommended for maltose estimations, and the results calculated as starch.

EXPERIMENTAL RESULTS

The mean rates of carbon dioxide absorption per hour in the six circuits of the assimilation apparatus for ten 3-day periods are shown in Table I. In five cases circuits 1, 4, and 6 were connected to empty assimilation chambers and circuits 2, 3, and 5 to chambers containing ears, and in the other five cases the positions were reversed. Analysis of variance of this data was used to determine the standard errors appropriate to the assimilation values. The mean

TABLE I

Mean Hourly Rates of Carbon Dioxide Absorption in the Six Circuits of the Assimilation Apparatus

Date 1946.	Total hours.	Controls (no ears)			Assimilating (with ears)		
		1.	4.	6.	2.	3.	5.
				mg. of CO_2.			
24/7 to 27/7	46·42	11·82	11·93	11·76	10·39	10·11	10·33
30/7 ,, 2/8	45·58	11·73	11·98	11·63	10·21	10·12	10·08
2/8 ,, 5/8	45·58	11·98	12·07	11·98	10·87	10·28	10·35
8/8 ,, 11/8	44·58	11·63	11·84	11·64	10·96	10·17	10·56
11/8 ,, 14/8	43·92	11·31	11·31	11·36	10·47	10·46	10·19
		2.	3.	5.	1.	4.	6.
14/8 ,, 17/8	43·83	11·69	11·23	11·44	10·13	10·83	10·07
17/8 ,, 20/8	42·92	11·92	11·23	11·44	10·28	11·40	10·82
20/8 ,, 23/8	42·25	12·00	11·42	11·87	11·15	11·14	11·76
23/8 ,, 26/8	41·83	11·45	11·25	11·26	11·33	11·19	11·94
26/8 ,, 29/8	41·17	11·21	11·69	11·07	11·53	11·44	11·38

Mean 11·62±0·09 10·78±0·09

Standard error of the means of 30 values 0·13

,, ,, ,, ,, 3 ,, 0·40

rates per hour of carbon dioxide absorption over a 30-day period in the control experiments and in the experiments in which ears were introduced into the chambers were respectively 11·62 mg. and 10·78 mg., with a standard error of 0·09 on these means of 30 estimates. The mean rate of assimilation over the whole time ears were assimilating was 1·29 mg. per ear per hour, and this figure is subject to an error of 0·13, or about 10 per cent. The mean rates of carbon dioxide emission by night for seven experiments are shown in Table II. Over a period of 21 days the rate of carbon dioxide output per hour per ear was 0·44 mg. with a standard error of 0·03, or about 7 per cent.

TABLE II

Mean Hourly Rates of Carbon Dioxide Emission by Night in the Three Respiration Circuits

Date 1946.	Total hours.	1.	2.	3.
21/7 to 24/7	24·42	0·275	0·202	0·162
24/7 „ 27/7	25·08	0·287	0·286	0·284
30/7 „ 2/8	26·33	0·764	0·841	0·562
2/8 „ 5/8	26·08	0·688	0·485	0·273
5/8 „ 8/8	26·42	0·592	0·891	0·802
14/8 „ 17/8	28·08	0·339	0·280	0·319
20/8 „ 23/8	28·25	0·261	0·188	0·165

Mean 0·426±0·025

Standard error of the means of 30 values 0·022

„ „ „ „ 3 „ 0·060

The hourly rates of assimilation and respiration for each 3-day period from the time of ear emergence until the ears were no longer green (21/7 to 23/8) are shown in Fig. 3. The mean hourly rate of assimilation remained practically constant at about 1·45±0·40 mg. of carbon dioxide from the time of ear emergence for about 18 days and then fell off to zero during the ensuing 12 to 15 days as the ears ripened and the chlorophyll disappeared. The mean hourly rate of respiration by night rose as the ears emerged to a maximum of about 0·70±0·65 mg. per ear per hour, and then fell off to a low value of about 0·20 mg. per ear per hour. In addition to these values estimates of the day-time respiration were made on two occasions, during the periods 2/8 to 5/8 and 17/8 to 23/8, by measuring the carbon dioxide emission by day of darkened ears kept otherwise under the same conditions as prevailed for the concomitant measure of assimilation. The values recorded were respectively 1·01 and 0·79 mg. per ear per hour, as compared with night respiration values of 0·48 and 0·32 mg. per ear per hour and net assimilation values of 1·50 and 0·78 mg. per ear per hour. The derived gross assimilation values of 2·51 and 1·57 mg. per ear per hour are also marked in Fig. 3. Finally the determinations made of the net assimilation rate of the flag leaf and its sheath during the 6 days preceding ear emergence and 3 days as the ear emerged are also indicated in Fig. 3 and show that over this time the amount of carbon dioxide assimilated by these organs was of the same order as that of the emerged ear.

The net uptake of carbon dioxide by the ear over the 33 days from 21/7 to 23/8 during which ears were assimilating was 622±65 mg. By calculation from the two estimates of respiration during the day the gross uptake was probably about 1,057 mg. per ear, during 496 daylight hours. The total emission of carbon dioxide by night was 117±7 mg. per ear during 296 dark hours. In terms of hexose this gives a net assimilation figure of 423 mg.

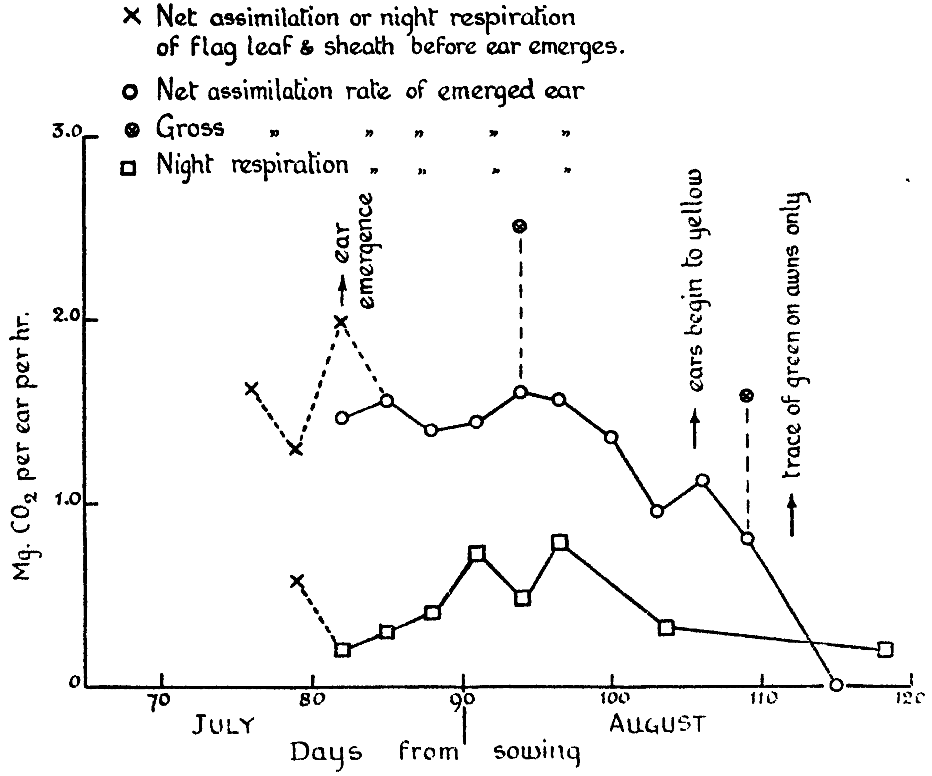

FIG. 3. Hourly rates per ear of gross assimilation, net assimilation, and night respiration in barley ears, during the period of ear development.

per ear due to photosynthesis in the ear and a net gain of hexose of 343 mg. per ear when allowance is made for the loss of carbon dioxide in night respiration. The gross assimilation figure is equivalent to about 700 mg. of hexose. The progress of accumulation of the products of photosynthesis in the ear is shown in Fig. 4 as running totals of the carbon dioxide absorbed, calculated as hexose, together with the dry weights of grain, and of awns, rachis, &c., and the starch content of the grain, determined at the end of each 3-day period on the ears whose assimilatory capacity had already been measured. The mean of 15 values for the dry weight of the ear at full ripeness was 1·30±0·05 g., of the grain 1·09±0·04 g., and for the starch content of the grain 649±35 mg., leaving 351 mg. per ear for other products accumulated in the grain, chiefly protein, and polysaccharides other than starch. It will be seen from Fig. 4 that after ear emergence only the grain increased in dry weight, the other organs of the ear reaching a maximum dry weight as the ear emerges. When the final dry-weight values are compared with the assimilation data it is found that the gross assimilation figure (i.e. the sum of the CO_2 absorbed by the ear and the CO_2 lost in day-time respiration as measured on darkened ears) is equivalent to 57 per cent. of the whole dry weight of the ear; the amount of CO_2 absorbed, or net assimilation, to 32 per cent.; and the net gain of hexose (i.e. net assimilation less CO_2 lost by night respiration) to 26 per cent.

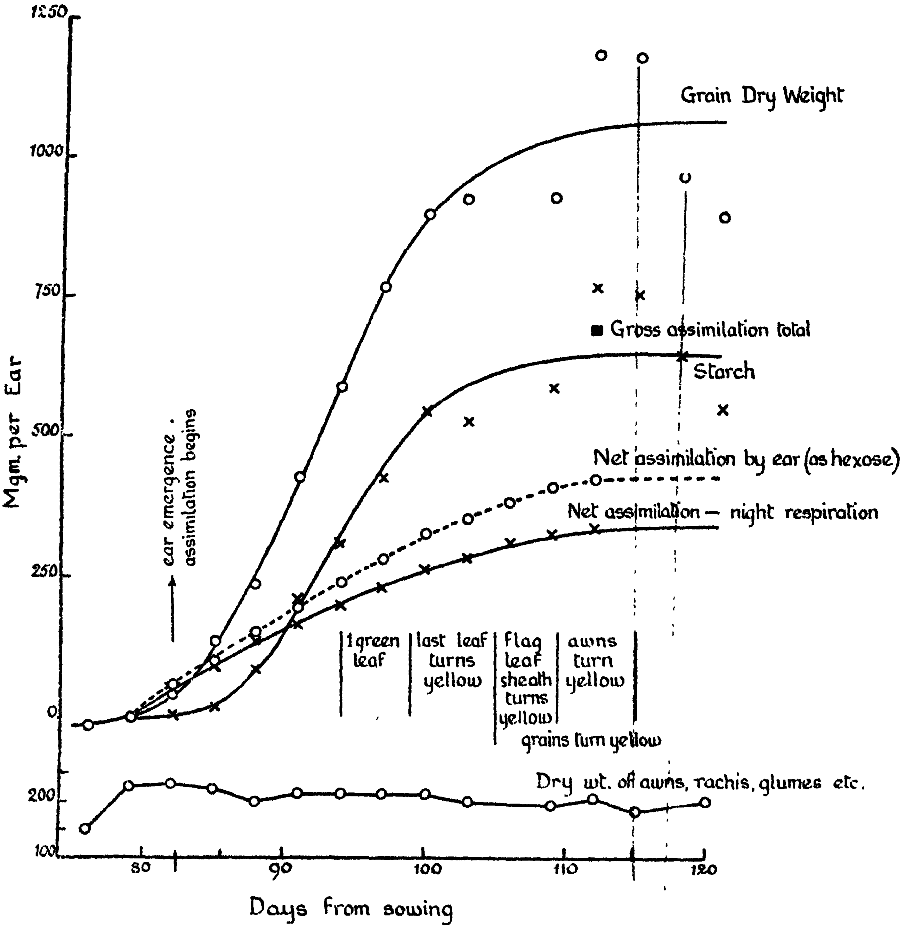

FIG. 4. Dry weights of grain, and of awns, rachis, &c., and the starch content of the grain of barley during ear growth, together with running totals of the carbon assimilated by the ear, expressed as hexose.

In terms of the amount of dry weight accumulated while the ear is assimilating, namely, 1,000 mg., the proportions are 70 per cent. for the gross figure, 42 per cent. for the net figure, and 34 per cent. for the net gain of hexose.

On the assumption that the substrate for respiration is provided by translocated sugar present in the ear at emergence together with sugar translocated during ear growth from other organs, then the gross assimilation by the ear could account for all the starch, while net assimilation is equivalent to 64 per cent. of the starch and the net gain in hexose to 52 per cent. On the other hand, the amount of non-starch products accumulated after ear emergence bears a close correspondence with the amount of hexose gained by assimilation in the ear, the figures being 351 mg. for accumulated non-starch material and 343 mg. for net hexose gain, so that the hypothesis that these compounds are in fact preferentially formed directly from the newly assimilated carbon must be considered as possible.

DISCUSSION

The data presented here afford confirmation of the results of earlier experiments with shaded ears, from which it was concluded that about 30 per cent. of the dry weight arose from autonomous assimilation, and show further that this contribution is made solely to the grain, there being no increase in the dry weight of other parts of the ear after its emergence. Since about 25 per cent. of the final dry weight of the ear is present at emergence, and 30 per cent. is contributed by its own assimilation, there remains about 45 per cent. to be supplied during ear growth by photosynthesis in other green organs. The assimilatory capacity of the flag leaf and its sheath together was found to be about equal to that of the emerged ear (see Fig. 3), and these organs remain green for about the same length of time as the ear. If, therefore, their assimilation rate also remains constant until the yellowing process begins, as does that of the ear, then the total contribution of the flag leaf and sheath will be of the same order as that of the ear, namely, 30 per cent., so that the bulk of the carbon not supplied at this time by the ear itself would be derived from this source. A number of earlier observations support this view; thus Archbold (1942) found that while removal of leaves only reduced the dry weight of the ear by amounts of 10 per cent. or less, additional removal of the flag-leaf sheath reduced the ear dry weight by 28 per cent. Watson and Norman (1939) record that shading either the ear or the rest of the shoot led to about equal reductions in the dry weight of the ear, indicating similar contributions by photosynthesis in the two cases, while Boonstra (1929) showed that in wheat only the top internode and peduncle, with their attached leaves, were effective in supplying material to the ear. Material for ear growth thus appears to be derived from assimilation in leaves and sheaths, before ear emergence, accounting for 25 per cent. of the final dry weight, by assimilation in the flag leaf and sheath, the top internode and peduncle after ear emergence accounting for 45 per cent. of the dry weight, the main contribution to this fraction coming from the flag-leaf sheath, and lastly by assimilation in the ear itself accounting for 30 per cent. of the dry weight.

Considering the accumulation of materials in the ear in more detail, inspection of Fig. 4 shows that as the ear emerges its net assimilation rate is for a short time sufficient to supply all the dry matter necessary for ear growth. As the rate of dry-weight accumulation increases, while ear assimilation rate remains constant, this phase is quickly terminated, and during the period of maximum growth rate large external supplies of carbohydrate are required. About 22 days after ear emergence, at which time growth rate begins to fall off, there is a period of 10 to 12 days in which the products of assimilation, though photosynthesis in the ear is declining, are again sufficient to supply the decreasing demands for growth. Rapid starch synthesis in the grain begins about 4 days after ear emergence, and the curve of starch increase has a sigmoid form similar to that of total dry weight, while the accumulation of non-starch materials (not separately shown in Fig. 4) proceeds at a nearly constant rate for about 23 days and then ceases. This fraction of the dry weight consists mainly of protein and carbohydrates other than starch (cellulose, &c.), and is collectively estimated as the difference between the total dry weight and the starch content. Both at the beginning and end of the growth period of the ear the net amount of autonomous assimilation is sufficient to supply the substrate for starch synthesis, but for a period of about ten days when starch is accumulating at the maximum rate the concomitant assimilation values fall short of the amount needed for starch synthesis.

It is of course impossible to deduce from the available data the precise fate of the carbon assimilated by the ear. As has been pointed out (p. 65), if only the final amounts are taken into account it would be possible, on the assumption that carbon dioxide respired during the day is at once reassimilated and converted to starch, for all the starch to arise from the photosynthetic activity of the ear. Moreover, in the barley ear sugar accumulates in the period before emergence and later declines to almost zero as the ear ripens. The amount of sugar accumulated is of the order of 100 mg. per ear and would thus be enough to supply the substrate for night respiration throughout the development of the grain, a process requiring about 80 mg. of hexose per ear. This hypothesis that carbon dioxide assimilated by the ear contributes to starch formation rather than to other products may possibly be supported by the observations of Harlan and Anthony (1920) that removal of the awns of barley diminished the dry weight of the ear by reducing the starch content of the grain, and of Watson and Norman (1939) and one of us (H. K. P. unpublished) that intake of nitrogen by the ear was little affected by shading this organ, so that reduction in dry weight when ears are prevented from assimilating falls upon the carbohydrate fraction. On the other hand, it is well known that detached leaves can form starch in the dark when given external supplies of sugar, so that it is not in all circumstances necessary, for starch synthesis, that assimilation shall be proceeding concurrently. As has been pointed out (p. 65), the close correspondence between the net gain in the ear by assimilation and the amount of non-starch products accumulated might suggest that it is indeed these materials which are preferentially formed from the autonomous assimilate.

It seems likely that organizing factors in the grain itself determine the nature of the products synthesized at any given time, irrespective of the nature and source of the carbohydrate supply. Thus in the early stage of ear development the products accumulated are mainly protein and cellulose type carbohydrates, and it may well be that all available carbohydrate supplies are directed to their synthesis, and there is in fact no discrimination between supplies arriving from organs other than the ear and those provided by the ear itself, as regards their use as substrates for formation of any of the required compounds. At present it can only be said with certainty that the ear is an important organ in contributing to the carbon supply for the grain, but the nature of the products to which it contributes remains a matter for speculation.

SUMMARY

A continuous record has been made of CO_2 assimilation by day and respiration by night of barley (var. Spratt Archer) ears attached to the plant, and kept under normal outdoor conditions. Assimilation proceeded for 33 days, during which 622 mg. of CO_2 per ear of average grain weight 1·09 g. were absorbed during 496 daylight hours and 117 mg. of CO_2 were emitted during 296 dark hours. Calculated in terms of hexose the net gain of carbon by autonomous assimilation was equivalent to 34 per cent. of the grain dry weight or 52 per cent. of the starch, a result in accordance with the estimates obtained in earlier experiments with shaded ears. On the assumption that sugars present in the ear at emergence or translocated to it during subsequent growth provide the substrate for respiration, the sum of CO_2 absorbed and the CO_2 formed by respiration in the day, as estimated on darkened ears, could account for 70 per cent. of the dry weight of the grain and all the starch.

About 30 per cent. of the grain consists of non-starch material, which might arise from the autonomous assimilate rather than starch. The fate of the CO_2 absorbed by the ear is discussed, and also the sources of the material required for its growth in addition to that supplied by its own assimilation.

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Phenol Oxidases in some Wood-rotting Fungi

BY

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INTRODUCTION

RECENTLY Preston and McLennan (1948) described the decolorization of dyes by certain types of wood-rotting fungi. They showed that white wood-rotting fungi would decolorize a series of dyes, whereas brown wood-rotting fungi had no such effect. The decolorization was not due to a change in pH, and was not an effect of a reversible oxidation-reduction system. The dyes were irreversibly changed, and it was suggested that 'they are oxidised by the enzyme systems of the white rots'. These authors described an experiment which indicated that oxygen is necessary for the decolorization of dyes by white rots, and it seems probable that an oxidase system is involved, as has been suggested in the Bavendamm reaction (Davidson et al., 1938; Boswell, 1941). By this reaction white wood-rotting fungi have been distinguished from brown wood-rotting fungi by the use of certain test substances added to the media. The most common of these are tannic and gallic acids. The darkening of these acids by the white rots is stated to be due to an enzyme of the catechol oxidase type. Brown rots do not cause darkening of gallic or tannic acid media. However, the dye decolorization as described gives a more reliable differentiation between the types of wood rots.

Many wood-rotting fungi give various 'oxidase' reactions (Boswell, 1941; Garren, 1938; Bose and Sarkar, 1937; Venkatarayan, 1936). Bose and Sarkar (1937), on account of the positive guaiacum reaction, reported the presence of laccase in wood-rotting Polypores. La Fuze (1937) showed that *Polystictus versicolor* (L.) Fr. acts on guaiacum, resorcinol, and tannin. *Polyporus betulinus* Fr. and *Fomes pinicola* Fr. had no action on guaiacum, but *Fomes pinicola* acted slightly on tyrosine. These and other similar tests indicate the presence of phenol oxidases.

The object of this work was to try to identify the agent responsible for the decolorization of the dyes. Only low concentrations of dye are decolorized, and the rate is always very slow. Preliminary experiments with some of the white rots indicated that the dye decolorization might be related to the presence of phenol oxidases. Extracts were prepared and tested on a number of substances, including neutral red and gentian violet, which were selected by Preston and McLennan (1948) as representatives of the dyes which white rots decolorize.

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EXPERIMENTAL AND RESULTS

Species of both types of wood-rotting fungi were selected. These were grown on 2·5 per cent. liquid malt medium in 250-ml. conical flasks for periods of 3–8 weeks at 24° C.

The action of a water extract of the mycelium of several different species on the various substrates is shown in Table I.

TABLE I

Oxidizing Enzymes in Wood-rotting Fungi

	White rots.							Brown rots.			
	Polystictus versicolor.	*Ganoderma applanatum.*	*Fomes robustus.*	*Fomes rudis.*	*Stereum hirsutum.*	*Poria sp. 2.*	*Polyporus gilvus.*	*Polyporus anthracophilus.*	*Polyporus Eucalyptorum*	*Coniophora cerebella.*	*Trametes lilacino-gilva.*
Guaiacum	+	+	+	+	+	+	+	+(–)	+(–)	–	–
Guaiacum + trace catechol	..	.	..	+	+	..	+	+	+	–	+(–)
Tyrosine	–	–	–	+(–)	+(–)	–	+	+	+	–	–
p-Phenylene diamine	+	+	+	+	+	+	+	+	+(–)	–	–
Hydroquinone	+	+	+	+(–)	+	+	–	+(–)	–	–	–
Pyrogallol	+	+	+	+	+	+	+	+	+	Λ	–
Phenol	+	+	+	+	+	+	–	+	–	Λ	–
Resorcinol	+	+	+	+	+	+	–	+(–)	–	Λ	–
p-Cresol	– (milky)	– (milky)	–	+	+(–)	–	+	+	+	–	–
m-Cresol	– (milky)	(milky)	– (milky)	–	–	– (milky)	–	+(–)	–	Λ	–
o-Cresol	+	+	+	+	+	+	+	–	–	Λ	–
Potassium ferrocyanide	+	+	+	+	Λ	+	–	–	Λ	Λ	Λ
Peroxidase	+	+	+	+	+	+	+	–	–	–	–
Catalase	–	+	–	–	–	–	–	+	–	–	–

+ indicates positive action
– indicates no action
+(–) indicates feeble action
Λ omitted

Where the action on guaiacum + trace of catechol was equal to the action on guaiacum alone no sign is given. A plus sign in this case indicates increased action.

Two brown rots (*Coniophora cerebella* Pers. and *Trametes lilacino-gilva* Berk.) showed no reaction on the substrates tested. Two other species (*Polyporus anthracophilus* Cooke and *Polyporus Eucalyptorum* Fr.) contain an enzyme which acts on a number of substrates including tyrosine. These reactions are similar to those described for the phenol oxidase 'tyrosinase' obtained from other sources (Nelson and Dawson, 1944). None of the brown rots gave a peroxidase reaction. Some white rots (*Polystictus versicolor* (L.) Fr., *Ganoderma applanatum* (Pers.) Pat., *Fomes robustus* Karst., and *Poria* sp. 2) contain a phenol oxidase which shows no action on tyrosine, but acts directly on guaiacum. Three species (*Fomes rudis* Berk., *Stereum hirsutum* (Willd.) Fr., and *Polyporus gilvus* Schw.) gave reactions which indicate the presence of more than one phenol oxidase.

Extracts of the white rots in most cases acted on the dyes to some extent, whereas extracts of the brown rots had no effect.

Examination of Table I suggests some possibilities regarding the enzyme concerned in the decolorization of the dyes. It seems obvious that the white rots contain a phenol oxidase differing from that which occurs in certain brown rots. The white rots contain also a strong peroxidase. Therefore, certain species of white rots were selected for further investigation. As *Polystictus versicolor* grew comparatively rapidly and was one of the most active species in decolorizing the dyes, it was most commonly used in the tests described. Some preparations were obtained also from *Ganoderma applanatum* and compared with those from *Polystictus versicolor*.

Preparation of phenol oxidase. Two methods were applied for the concentration and purification of the enzyme.

1. The mycelium was ground and extracted several times with water and the medium added to the extract. Acetone was added to a concentration of 50 per cent., the precipitate centrifuged off, and the remaining liquid increased to 70 per cent. acetone concentration. The precipitate from the 70 per cent. acetone was collected and dried. This method is similar to that used by Kubowitz (1937) in the first stages of his preparation of phenol oxidase. This product contained a phenol oxidase and gave a weak reaction for peroxidase. It acted on the dyes.

2. The majority of the enzyme preparations were made by the method of Dalton and Nelson (1939) without repeating the purifications. Ammonium sulphate was added to the aqueous extract until the solution was 0·6 saturated, the precipitate centrifuged off, suspended in water, and dialysed overnight. The solution was reprecipitated with the same concentration of ammonium sulphate, and the precipitate centrifuged, suspended in water, and dialysed against running water till free from ammonium sulphate. The phenol oxidase was adsorbed on to acid-washed kaolin at pH 5, with subsequent elution in phosphate buffer at pH 8·4. The eluate was dialysed against running water and finally against glass distilled water till almost free of phosphate.

The final preparations, unlike the original extracts, gave no peroxidase reaction. Though mostly adsorbed by the kaolin, peroxidase was not eluted by the phosphate. The final preparations acted on the dyes. This indicates that peroxidase was not a factor in the decolorization and the following observations support this conclusion:

1. Analyses of two preparations acting on the dyes gave the following results:

   Preparation from *Polystictus versicolor*: Cu, 0·15 μg./ml.; Fe, nil.
   „ „ *Ganoderma applanatum*: Cu, 0·5 μg./ml.; Fe, nil.

2. Peroxidase was prepared from horse-radish by the method of Keilin and Mann (1937). Solutions of this preparation tested over a pH range 4–6 caused no change in the dyes.

3. Some preparations of the white rots which gave a strong peroxidase reaction showed very little action on the dyes (e.g. *Polyporus gilvus*).

PHENOL OXIDASE

The preparations after elution and dialysis acted on the substrates given in Table I, and, therefore, contained a phenol oxidase. As indicated above, copper was present in these solutions.

1. *Decolorization of the dyes*

In order to investigate the relationship of the phenol oxidase to the dye destruction, concentrations of 0·001 per cent. of buffered dye were added to an equal volume of the enzyme preparation, and boiled controls prepared for comparison. Merthiosal (total concentration of 1 in 60,000) was added for prevention of bacterial growth. The tubes were incubated at 40° C.

The decolorization of the dyes by most of the extracts was very slow and often not complete. However, the change was quite definite when compared with the boiled controls which showed no apparent change over a period of several days. Solutions buffered at pH 4, 5, 6, and 7 all showed some action, pH 6 approximately being the optimum. Tests on the dyes were carried out at pH 6–6·1. The dyes changed very gradually over a period of several days, neutral red fading more rapidly than gentian violet.

The action of the enzyme on the dyes was tested in evacuated Thunberg tubes and compared with the action in air and with boiled controls. The dyes in the evacuated tubes remained the same colour as those of the boiled controls, while those in air changed gradually. The presence of oxygen is, therefore, required for the reaction. As mentioned previously, Preston and McLennan (1948) showed this with white rots grown on agar slopes.

The composition of the buffer affected the type of dye-change by the enzyme and concentrated buffer caused a slowing of the reaction. In general, phosphate buffer M/15 diluted 4 times was added. More concentrated buffer not only slowed the reaction but caused the gentian violet to develop a bright pink colour in the first stages of the change.

The action of the enzyme on catechol varied also with the type of buffer, as shown below. The uptake of oxygen of two preparations was measured, using equal amounts of two different buffers, pH 6·1.

	c.mm. O_2/ml./30 min.	
Preparation.	Phosphate buffer M/15.	Phosphate-citrate buffer (Sörensen).
Polystictus versicolor	27·3	17·5
Ganoderma applanatum (before purification by adsorption)	102	68·6

Similar effects of different buffers on other phenol oxidases have been reported (Boswell and Whiting, 1938).

The rate of paling of the dyes was related to the amount of enzyme preparation added. A high concentration of enzyme was always necessary for complete decolorization of the dyes. Small amounts failed to destroy completely the dyes even over periods of 2 weeks. Enzyme solutions remained active to some extent throughout this period, as shown by the addition of other substrates at the end of the test.

Concentration of the enzyme preparations in front of a fan at room temperature had a varying effect. In certain cases some activity was lost. Other preparations after concentration by this means showed a more rapid action on the dyes.

TABLE II

Test solution.	Original preparation.	Preparation concentrated 5 times.
1. 1 ml. enzyme 1 ml. 0·001% neutral red	Decolorized 4 days	Decolorized 20 hours
2. 1 ml. enzyme 0·5 ml. 0·001% gentian violet	Changed, but not decolorized in 4 days	Almost decolorized in 4 days
0·5 ml. enzyme added to 2	Colour still present 12 days later	Colour disappeared 2 days later

Attempts were made to detect uptake of oxygen during the dye-change. The action was so slow that results were considered unsatisfactory.

A series of estimations of uptake of oxygen were carried out on different preparations using catechol as substrate. At the same time the decolorization of the dyes by these preparations was followed qualitatively by comparison with boiled controls.

The reaction mixture for oxygen uptake consisted of buffer, 1 ml.; water, 1 ml.; enzyme, 1 ml.; catechol, 0·5 ml. of 2 per cent. solution added from the side arm. The experiments were carried out at 40° C.

TABLE III

	Preparation.	c.mm.O_2/ml./30 min.	Dye decolorization.
Compared together	*Polystictus versicolor* (1)	18·7	Dyes attacked slowly. Colour still definite in 6 days
	Polystictus versicolor (2)	31	Dyes attacked more rapidly. Colour much paler than in (1) in 6 days
Compared together	*Ganoderma applanatum* (1)	34·6	Dyes slowly attacked. Colour still present in 9 days in neutral red and gentian violet
	Polystictus versicolor (3)	70	Neutral red paled in 1 day, very slight colour after 4 days. Gentian violet paled, but colour present less degree than above in 9 days
	Ganoderma applanatum (2) (before adsorption on to kaolin)	102	Neutral red—change rapid, complete in 4 days. Gentian violet—more rapid change. Colour distinctly paler than above in 9 days

The changes produced in the dyes, whether complete or partial, appeared to be irreversible. Attempts to restore the colour by treatment with various reagents all failed.

A decolorization of dyes, similar to the enzymic effect, can be produced by hydrogen peroxide. Tubes containing 1 ml. of 0·001 per cent. neutral red or gentian violet were decolorized slowly when hydrogen peroxide was added to a concentration of 0·08 per cent. The dyes were attacked over a range of pH from 3 to 6, pH 6 being the optimum.

Some oxidation systems produce hydrogen peroxide and the possibility of its formation as a factor in the dye decolorization was investigated. Catalase, prepared by the method of Sumner and Dounce (as described by Sumner and Somers, 1947), was added to solutions of the dye and enzyme, the amount being sufficient to destroy comparatively large quantities of hydrogen peroxide. The presence of the catalase had no effect on the rate of decolorization of the dyes. The catalase was present at the end of the experiment, as indicated by the addition of hydrogen peroxide.

The slow rate of reaction on the dyes suggests that the products may act as inhibitors of the enzyme. Solutions of the dyes and enzyme were incubated as described, and, after 15 hours, when there was noticeable change in the colour of the dye, these solutions were divided into two equal volumes. One volume was dialysed for 24 hours, in order to remove soluble degradation products. A further amount of dye was added to both volumes and the tubes incubated. The rate of decolorization of the second volume of dye was equal in both cases. In neither the case of neutral red nor of gentian violet was an inhibitory action of breakdown products demonstrated by this means.

2. *Further properties of the phenol oxidase*

Effect of carbon monoxide. Enzyme preparations were treated with carbon monoxide, both in the light and in the dark. The action on the phenolic substrates and the dyes was not affected.

Effect of cyanide. Potassium cyanide M/1000 at pH 6 was added to the enzyme preparation. Decolorization of the dyes took place more slowly in the presence of cyanide. The action on other substrates, including catechol, was also slower in the presence of cyanide.

Effect of metallic ions. Salts of copper, iron, zinc, manganese, and magnesium had no effect on the dye decolorization by the enzyme. Under certain conditions gentian violet was decolorized by copper salts alone, but this change was reversible and the colour reappeared on standing. In the presence of the enzyme copper salts showed no such effect. The rate and amount of decolorization by the enzyme was not increased by the copper salts.

Effect of organic acids. Solutions of malic, citric, and fumaric acids at pH 6 showed no effect on the dye decolorization by the enzyme preparations.

Effect of catechol. Catalytic amounts of catechol, when added to the enzyme solutions, caused no increase in the rate of dye destruction.

Action on amino-acids. Certain phenol oxidases, in the presence of catalytic amounts of catechol, will convert amino-acids and amines into highly coloured compounds. Preparations from *Polystictus versicolor* and *Ganoderma applanatum* gave a red colour with glycine or di-methylamine, in the presence of catechol.

Preparations from these white rots acted directly on adrenalin, forming a red-coloured compound. However, the reaction was given more strongly by preparations from *Polyporus anthracophilus*, a brown rot.

Oxidation of tannic and gallic acids. Tannic and gallic acids (Bavendamm) buffered at pH 6 were added to the enzyme preparation and incubated at 40° C. The darkening of these acids started almost immediately and was distinct in an hour. The colour became intense overnight.

Peroxidase from horse-radish acted on these substrates very slightly and very slowly. The enzyme of *Polyporus anthracophilus* slowly attacked gallic and tannic acids.

Laccase from *Rhus succedanea.* Crude preparations of laccase from *Rhus succedanea* were obtained by treating the latex with acetone, centrifuging, and drying the precipitate. Solutions of these preparations acted on the various substrates described for laccase (Yakushigi, 1940; Gregg and Miller, 1940; Sumner and Somers, 1947). Some preparations acted very slightly on resorcinol and *o*-cresol, and others showed no reaction on these substrates. Gallic and tannic acids were oxidized by this enzyme. However, there was no action on the dyes even in high enzyme concentrations.

Phenol oxidase of brown rots. Four brown rots were examined for their phenol oxidase content. Of these, *Polyporus anthracophilus* and *Polyporus Eucalyptorum* acted on a number of phenolic substrates, including tyrosine (Table I). *Coniophora cerebella* and *Trametes lilacino-gilva* showed negligible action on the substrates tested.

Extracts of *Polyporus anthracophilus* were prepared as described for the white rots. These acted on the phenolic substrates as shown, but did not attack the dyes. As indicated already, gallic and tannic acids were slowly oxidized.

Discussion

Consideration of the above results indicates that the destruction of the dyes by the white rots is associated with the presence of a specific phenol oxidase. The preparations analysed contained copper but no iron. The slowing of the action on the dyes by cyanide is in accordance with the inhibitory effect of cyanide on phenol oxidases from other sources. Keilin (1929), Keilin and Mann (1938), and others have shown that the polyphenol oxidases from potato and mushroom are inhibited by potassium cyanide. They showed also (1939) that the laccase of *Rhus succedanea* was inhibited by cyanide, and Gregg and Miller (1940) obtained a similar result with laccase from the wild mushroom.

The increase of dye destruction with increased rate of catechol oxidation also indicates a close relationship with a phenol oxidase. The action on the dyes is very slow compared to the oxidation of the phenolic substrates and this might be due to the formation of some inhibitory substance. However, dialysis after some decolorization had occurred had no effect in hastening the decolorization of further amounts of dye. If inhibitory substances are formed they are not removed by dialysis. There is no evidence for an enzyme system involving other substances such as catechol. Catalytic amounts of catechol did not increase the rate of dye destruction, and, moreover, all soluble constituents would be removed from the enzyme solution during the long dialyses. The dyes and potassium ferrocyanide differ entirely in constitution from each other and from the phenolic substrates, and the enzyme, therefore, may have various modes of attack. Further examination of the dye-decomposition products is necessary to show what is involved in the decolorization by the enzyme.

The white rots examined all contain a phenol oxidase. Some, according to their actions, may contain a mixture of phenol oxidases. There is a great variation in the rate of dye destruction in the several species tried, and this is possibly due to the amount of enzyme produced. *Polyporus gilvus*, which showed a very weak action on the phenolic substrates, had a negligible effect on the dyes, whereas *Polystictus versicolor*, which acted strongly on the phenolic substrates, had a marked effect on the dyes.

The rate of dye destruction on agar slopes is very slow compared with the oxidation of catechol, indicating also that high concentrations of enzyme are necessary for the decolorization. The enzyme preparations were weak in their action compared to the mycelium on agar slopes. This is probably due to the fact that complete extraction of the enzyme was never achieved, and a great deal of activity lost in the process of purification.

The phenol oxidase which decolorizes the dyes acts strongly on gallic and tannic acids and is, therefore, also responsible for Bavendamm's test which is given so readily by the white rots.

The phenol oxidase of the white rots is more closely related to 'laccase' than to 'tyrosinase'. This is indicated by its substrate action and by the fact that it is unaffected by carbon monoxide. Keilin (1929) and Keilin and Mann (1938) showed that polyphenol oxidases, often termed 'tyrosinase', are inhibited by carbon monoxide whereas the laccase of *Rhus succedanea* (Keilin and Mann, 1939) is unaffected. Laccase from the wild mushroom (Gregg and Miller, 1940) is reported to oxidize potassium ferrocyanide and this action was given by the white rots. Laccase from *Rhus succedanea* also acted on gallic and tannic acids. However, unlike the phenol oxidase in the white rots, it showed practically no action on resorcinol or on *o*-cresol and did not attack the dyes. Yakushigi (1940) prepared laccase from *Lacterius piperatus* and showed that it acted on resorcinol, but not on *o*-cresol. It is evident that these related enzymes from different sources show a considerable variation in their substrate specificity.

Some brown rots, according to their reactions, contain an enzyme more closely related to 'tyrosinase', but brown rots, as shown by Preston and

McLennan (1948), do not decolorize the dyes. *Polyporus anthracophilus* gave very strong tyrosinase reactions, and in concentrated preparations showed no action on the dyes. In some other brown rots, e.g. *Coniophora cerebella*, a potent rot, phenol oxidase, if present, is not in an active form.

The presence of phenol oxidases in wood rots has been associated with a variation in the type of rot produced (Campbell, 1930; Boswell, 1941; Cartwright and Findlay, 1944), and, in the small field examined, there is distinct difference in the distribution of these enzymes between the white and the brown rots. Boswell and Whiting (1938) and Baker and Nelson (1943) have shown that the phenol oxidase system is concerned in the respiration of the potato tuber. This function was further investigated by Boswell (1945) and by Robinson and Nelson (1944), and Walter and Nelson (1945) have shown that tyrosinase has the role of a terminal oxidase in the sweet potato. A further study of these phenol oxidases in the wood rots might indicate whether or not they play such a part in these fungi.

SUMMARY

1. The decolorization of dyes by white wood-rotting fungi is attributed to a specific phenol oxidase. Brown rots, on the other hand, cause no decolorization and do not contain this enzyme in significant amounts.

2. Some properties of the phenol oxidase of white rots have been studied and its action is shown to be similar in certain respects to that of laccase, though the substrate specificity differs.

3. Some brown rots contain a phenol oxidase which, according to its reactions, resembles tyrosinase of mushroom and potato. Other brown rots examined showed a negligible phenol oxidase reaction.

I wish to express my thanks to Professor J. S. Turner and Associate Professor E. I. McLennan of the Botany School for suggesting this problem and for their helpful advice, and to Professor V. M. Trikojus for his criticism of the paper. My thanks are due also to officers of the Council for Scientific and Industrial Research for some of the analyses, and to Miss Barbara Dale for valuable technical assistance.

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Changes in the Drought Resistance of Wheat Seedlings during Germination

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With four Figures in the Text

IT is generally agreed that dormant embryos are extremely resistant whereas seedlings are relatively susceptible to desiccation; the stages of germination therefore provide an organized plant system which during development displays rapidly decreasing drought resistance. A study of the reaction of seedlings to desiccation during these different stages should thus clarify some aspects of drought resistance. It was the aim of the present investigations to make a preliminary survey of such behaviour; primarily, to ascertain the rate of change in drought resistance of dormant wheat embryos from germination onwards, and to correlate such changes with stages in seedling development.

METHODS

Wheat seedlings were germinated and grown on stainless-steel wire gauze supports. Each support consisted of a square of 12-mesh gauze, suspended from glass stands by stainless steel wire, and held 12 grains in meshes which had been suitably enlarged. The height of each support was adjusted so that the basal one-third of the grain was immersed in a nutrient solution contained in glass dishes. At appropriate stages of germination a number of the seedlings were removed for drying. The number of seedlings was reduced to 8 of uniform size per gauze. The gauzes were then randomized for treatments and suspended over concentrated sulphuric acid in desiccators. At suitable intervals the gauzes were removed from the desiccators, half of the seedlings taken out at random for determination of moisture content, and the remainder immersed in a solution for recovery.

Before determining moisture contents the embryos were separated from the endosperms with the aid of a dissecting microscope. In expt. 1, moisture contents were obtained after drying at 95° C. for 24 hours. In expt. 2, the plant material was placed in small weighing-bottles on an electrically heated wire mat maintained at 50° C. All weighings were made on a Bunge microbalance.

The remaining seedlings were kept fully immersed in solution for 24 hours in order to regain turgidity; the root and shoot lengths were then measured and the seedlings grown for a further 8 days. Measurements of shoot and root

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[1] This work was carried out while the author held a Farrer Memorial Scholarship. It formed part of a thesis submitted to the University of London for the Ph.D. degree.

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length were made at intervals of 2 days and the amount of growth made over 8 days taken as a measure of resistance to drying. The technique of estimating drought resistance by measuring the resumption of growth after a period of drought was first used by Ashby and May (1941).

RESULTS

Experiment 1

Two Australian varieties—Bencubbin and Charter, which are reputed respectively to be drought-resistant and drought-susceptible—were subjected to different degrees of desiccation at stages of growth corresponding to 3, 5, 9, and 17 days from the beginning of soaking. The degrees of drying were those which occurred when the seedlings were suspended in desiccators over concentrated sulphuric acid at a temperature of 19·3° C. for 3, 9, 27, and 81 hours.

Germination, growth, and recovery from drying were all carried out in a 0·002 M $CaCl_2$ solution. The 3-, 5-, and 9-day plants were kept throughout in diffuse daylight of a laboratory at a temperature of 14–15° C.; the 17-day plants were grown under these conditions for 12 days and were then removed to a greenhouse exposed to full winter daylight and with a temperature of 3–12° C. The plants remained here for the last 5 days of pre-drying growth and for the growth period following recovery. As no significant differences could be detected between the two varieties, means only for varieties are presented in the results.

TABLE I

Size of Plants at Time of Desiccation

Age (days).	Shoot length (mm.).	Total root length (mm.).	No. roots per plant.	Dry wt. embryo (mg.).
3	3	9	3	1·5
5	11	41	4	4·2
9	38	75	6	15·0
17	94	95	7	27·4

The sizes of seedlings at the times of desiccation are shown in Table I. Drying for the same period of time resulted in different relative amounts of water loss from the seedlings of different ages (Table II), but an extremely wide range of desiccation was obtained in all the sets.

The time of onset of wilting could not be observed accurately in the short coleoptiles of the 3- and 5-day-old plants. In the 9- and 17-day plants incipient wilting was observed after about 3 or 4 hours of drying. Most of the stomata were then closed, although a few remained permanently open. Stomata of 9- and 17-day plants dried for 81 hours did not reopen even after 2 days' immersion following drying. The shoots in these treatments did not regain turgidity during this period, although when kept for several days longer with the roots only in water, elongation of the second leaf, which was in the primordial stage at the time of desiccation, was observed. The second leaf initial did not appear to be damaged by this severe desiccation and

TABLE II

Shoot and Root Growth made by Seedlings of Different Ages after Recovery from Drying to the Moisture Contents shown

Age (days).	Hours of drying.	Water content after drying (% dry weight).	Percentage water lost on drying.	Growth made on recovery (mm. in 8 days).	
				Shoot.	Root.
3	0	450	0	63	77
	3	180	60	51	48
	9	36	92	40	28
	27	10	98	13	4
	81	4	99	2	0
5	0	530	0	68	55
	3	333	35	64	39
	9	168	66	45	23
	27	47	91	35	7
	81	5	99	4	0
9	0	670	0	57	44
	3	452	32	51	7
	9	222	67	42	7
	27	73	88	30	5
	81	31	95	0	0
17	0	675	0	59	21
	3	413	39	40	8
	9	228	66	31	7
	27	77	88	19	0
	81	15	98	0	0

sufficient water was absorbed through the roots or mesocotyl to allow its elongation to continue.

The plumules of the 3- and 5-day plants dried for 81 hours regained turgidity and slight elongation continued. Plumules so treated were very corrugated as if cells in localized areas were permanently injured, while in others they had regained their previous turgid state and continued to elongate. The cells in some areas were flattened and buckled, the normal shape and volume not being regained. A similar corrugated appearance was noticed in a few areas on the leaves of the 9- and 17-day plants dried for 27 hours.

Both root and leaf cells were folded and wrinkled after drying for about 9 hours in the 9- and 17-day plants, and after about 30 hours in the 3- and 5-day plants. Coagulation of the protoplasm did not appear until the plants had been dried for a longer period. Folding of the cell-walls seemed to be more severe in leaf tissue than in root-cells. Root-hairs of plants of all treatments no longer showed folding of cell-walls after immersion for a few minutes in water. The roots in all treatments, even those dried for 81 hours, became turgid within a few minutes of replacement in water, whereas the leaves and coleoptiles of the 9- and 17-day plants dried for 81 hours did not become turgid even after 2 days in water. However, this apparent resumption of turgidity by the roots was not followed by further growth.

The shoots of seedlings of all ages dried for 3 and 9 hours regained their original moisture contents within 24 hours of immersion in 0·002 M $CaCl_2$ solution, but this was not true of seedlings dried for longer periods. However, recovery in all treatments was taken as being completed at 24 hours after replacing in the solution and this time was used as the datum line for purposes of growth measurements. Shoot-growth of undroughted plants in this experiment was approximately linear, so arithmetic differences of the lengths at the end and beginning of the growth period following recovery were taken as measures of growth.

The roots of 3- and 5-day old plants dried for a period of 3 hours grew at a slower rate than roots of undroughted plants, and those dried for 9 hours grew even more slowly (Table II). There was a lag period of 2 days in the latter before growth was resumed. Roots of plants droughted for 27 hours did not recommence growth until about 6 days after recovery. Measurements of the separate roots were not made in this experiment, so it is impossible to state if roots existing at the time of drying were permanently injured (cf. expt. 2).

Experiment 2

Seedlings of one variety (Bencubbin) at stages of 1, 3, 5, 7, and 9 days from the commencement of soaking were dried in desiccators containing concentrated sulphuric acid for periods of 0, 2, 5, 10, and 30 hours. The entire experiment was carried out in the diffuse light of a laboratory at a temperature ranging from 8° to 12° C. Seedlings were germinated and grown throughout with daily changes of a culture solution containing (in p.p.m.) 252 $Na_2HPO_4 \cdot 12\ H_2O$, 100 $CaCl_2$, 185 K_2SO_4, 910 $NaNO_3$, 125 $MgSO_4 \cdot 5\ H_2O$, 279 $Fe_2\ (C_4H_4O_6)_3 \cdot H_2O$, and adjusted to pH 5·5.

The sizes of seedlings at the times of desiccation are presented in Table III and are shown diagrammatically in Fig. 2. Seedlings of 1 day corresponded to the time of rupture of the pericarp.

TABLE III

Size of Embryos at Time of Desiccation

Age (days).	Shoot length (mm.).	Total root length (mm.).	No. roots per plant.	Dry wt. (mg.).
1	2	—	—	0·60
3	3	5	3	0·90
5	5	19	3	1·52
7	8	37	3	2·87
9	21	88	5	4·88

The relative rates of moisture loss during drying were similar for the 1-, 3-, and 5-day plants resulting in different absolute values, but there was a slower rate of loss from the 7- and 9-day plants (Table IV). Roots lost moisture much more rapidly than shoots, as shown by the data for 9-day treatments in which the moisture contents of roots and shoots were deter-

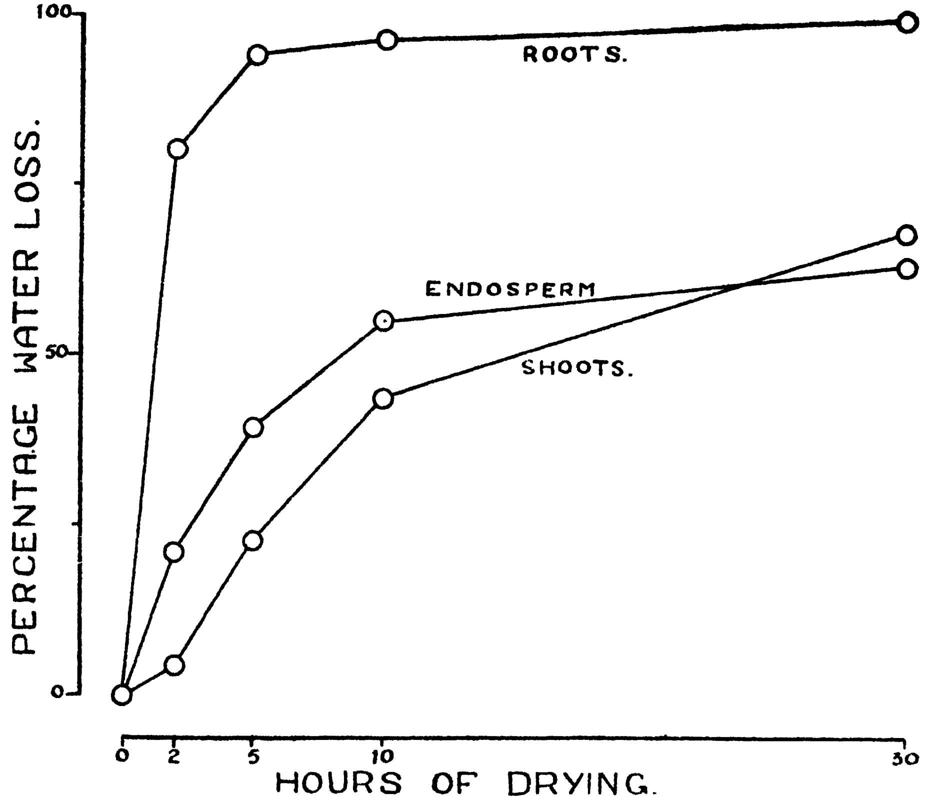

FIG. 1. Percentage loss of moisture by roots, shoots, and endosperm of 9-day-old plants after drying for different periods over concentrated sulphuric acid.

mined separately (Fig. 1). The much more rapid desiccation of the root than of the shoot may be attributed to the different surface/volume relationships of the two tissues and the absence of cuticle in the root. The data given in Table IV, therefore, do not give a correct picture of the moisture contents of the roots in the various treatments. Because of the low proportion of root to shoot, the values of moisture content of the whole embryos nevertheless correspond very closely to those of the shoots alone.

TABLE IV

Moisture Contents of Seedlings of Different Ages after Drying for Varying Periods (% dry wt.)

Age (days).	Hours of drying.				
	0.	2.	5.	10.	30.
1	294	163	53	17	8
3	531	270	120	41	8
5	637	256	108	74	13
7	756	407	245	157	57
9	784	549	372	266	149

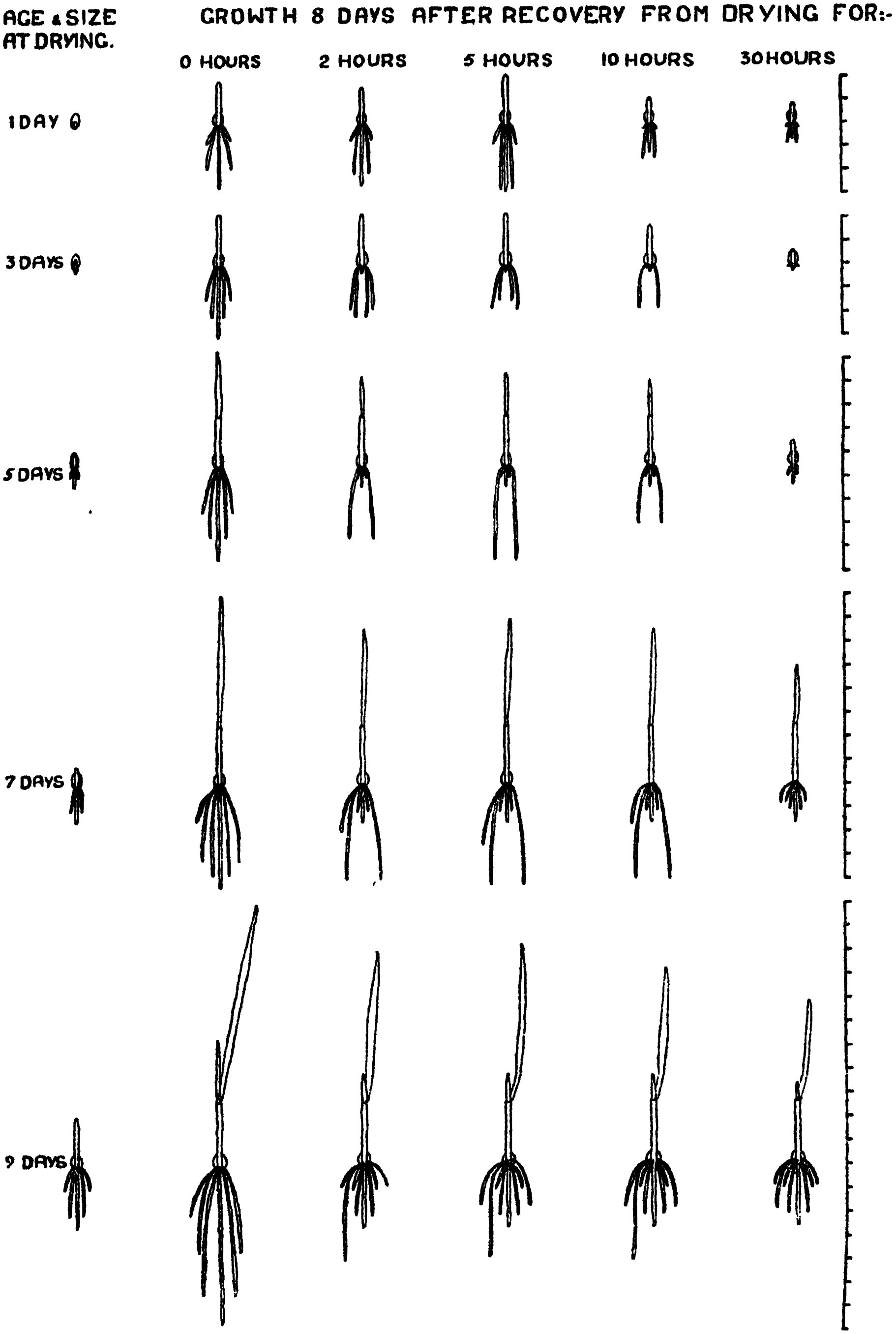

FIG. 2. Diagram showing mean lengths of different organs of seedlings (coleoptile, 1st and 2nd leaves, 1st root, 2nd and 3rd roots, and 4th, 5th, and 6th roots) at time of desiccation, and the lengths of these organs after 8 days' growth following drying for varying periods. (Each division on scale equals 1 cm.)

The amount of growth made in 8 days by the component organs of seedlings following recovery from drying was determined (Fig. 2). Roots of 2 mm. or more in length were completely killed by the least severe desiccating treatment, which probably represented a loss of moisture of about 80 per cent. Root initials, however, were not damaged even when the embryos had lost about 98 per cent. of their moisture. Roots 1–2 mm. in length (cf. 3-day series) were retarded in growth when the embryo had lost 77 per cent. of its moisture and were completely killed by a loss of 92 per cent. of the moisture. The effect of severe desiccation on the root initials was to lengthen the time before the young root appeared; once growth recommenced it continued at a rate comparable with that of undroughted plants.

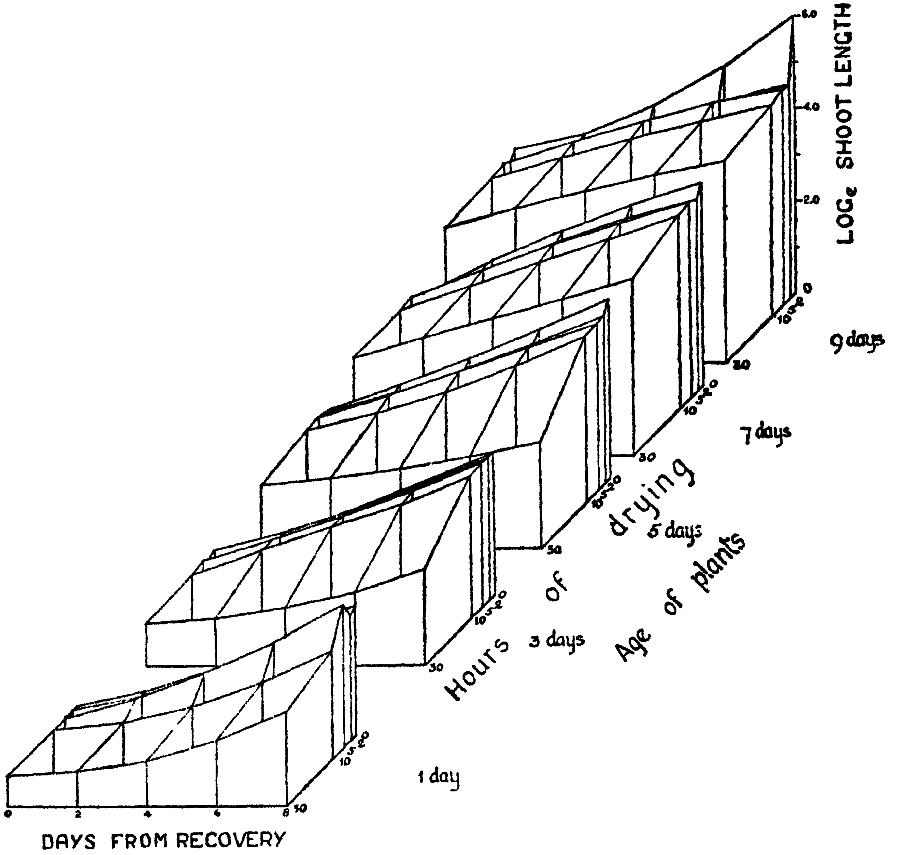

FIG. 3. Series of five diagrams of three-dimensional models showing logarithms of shoot-length of seedlings at 2-day intervals following recovery from drying for varying periods over concentrated sulphuric acid. Each model shows all treatments within one age-group; the values used in the diagrams were calculated from second-order equations fitted to the observed values for each treatment.

The data of lengths of shoot, after transformation to logarithms, were subjected to analysis of variance. The interactions with time from recovery were partitioned by the use of orthogonal functions (Cochran, 1939). The interaction of chief interest is that of 'age at drying × hours of drying × times of recovery after drying'; this showed significant differences in both the linear and quadratic terms. These differences are shown in Fig. 3, in which are presented the second-order regression equations of log. shoot length on time from recovery. The significant positive quadratic term indicated that elongation, particularly of undroughted plants, proceeded at a rate greater than exponential. Seedlings up to 5 mm. in length were not greatly affected by drying for 5 hours or less (i.e. with a loss of less than 80 per cent. moisture); more severe drying caused a lag in resumption of the initial growth rate, but this rate was resumed in the 1-day plants. In the older seedlings, however, the growth rate was retarded over the whole period and there was no indication of the former growth rate being resumed. The reduction in growth rate was shown even by the least-severely dried plants, but there were no significant differences between the 2-, 5-, and 10-hour treatments. Drying for 30 hours in the older seedlings (5-, 7-, and 9-days) resulted in further reduction in the growth rate.

A comparison of the relative growth rates with water-content indicated that the 1- to 7-day seedlings showed a marked reduction in growth with a loss of about 85 per cent. of the moisture. The growth of the 9-day seedlings was greatly retarded by a much smaller water loss. In general, the older the seedling the less water was lost before a reduction in growth rate was evident.

DISCUSSION

The early stages of growth of wheat seedlings showed three periods during which their behaviour after drying differed greatly. The first phase (A, Fig. 4) covered the time from the beginning of soaking to the stage when the coleoptile was about 3–4 mm. long. During this period there was a rapid absorption of water in the first hour, the water-content rising from *c.* 8 per cent. to *c.* 120 per cent. on a dry-weight basis. This probably represented imbibition by pre-existing cells of the embryo. The absorption rate, which had fallen after the first hour, gradually increased after about 4 hours and became very rapid up to the time of rupture of the pericarp. This high absorption rate continued until the moisture content was about 550 per cent. dry weight. This period probably included the time when most of the coleoptile cells were laid down (Avery and Burkholder, 1936), and during the whole of the period most of the plumule cells must have been in a meristematic state. The primary root appeared soon after the rupture of the pericarp and at the end of the period A was 3–4 mm. long. Severe drying at the end of period A killed the longer roots, but did not permanently injure the plumule. A loss of 85 per cent. or more of the moisture during this phase caused a lag in resumption of growth; but growth was gradually resumed and finally proceeded at a rate comparable with undroughted plants. This check in growth following severe desiccation suggests a dislocation in cellular organization, but the subsequent resumption of growth at the original rate indicates that it was only of a temporary nature. Wheat seedlings during this stage, therefore, may be regarded as being entirely drought-resistant.

The second phase of growth in relation to seedling behaviour after desiccation (B, Fig. 4) embraced the period of elongation of the coleoptile to the time of emergence of the first leaf. Cell-division probably continued during the early part of this period, but most of the elongation is likely to have resulted from extension of pre-existing cells in the basal region of the coleoptile (Avery and Burkholder, 1936). The rate of water absorption during this period was decreasing, but the water-content rose from about 550 to 800 per cent. dry weight. Slight drying resulted in death of emerged roots but did not injure the initials, which gradually grew out and expanded at a rate comparable with those of undroughted plants. Throughout this period there was a decrease in the growth rate of plants which had been subjected to equal degrees of desiccation. A loss of 60 per cent. of total water caused a slight reduction in the growth rate and this effect became more pronounced with increased desiccation. Loss of 98 per cent. of the total moisture, however, did not permanently injure the plant.

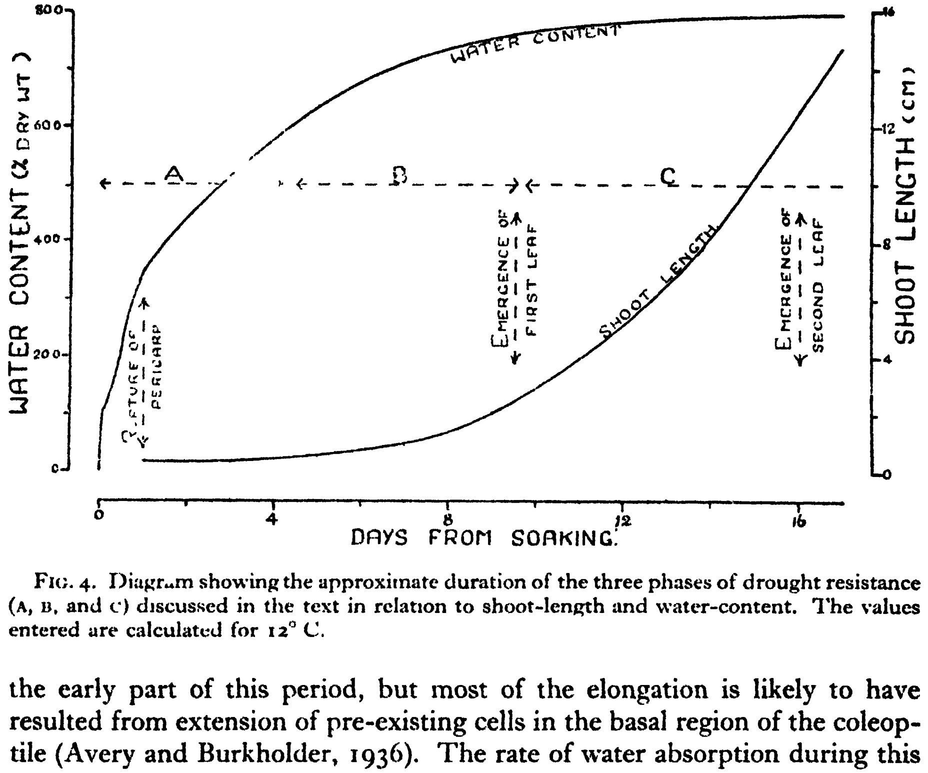

FIG. 4. Diagram showing the approximate duration of the three phases of drought resistance (A, B, and C) discussed in the text in relation to shoot-length and water-content. The values entered are calculated for 12° C.

Period C (Fig. 4) extended from the emergence of the first leaf to the end of the experiment. During this phase the moisture content remained fairly constant. Root reaction to drying resembled that in period B. The expanded tissue during this period was permanently injured by a loss of 92 to 98 per cent. of the total moisture and did not regain turgidity when immersed in water. Leaf primordia, however, were not killed and it is believed that under suitable conditions both root and leaf tissue would be regenerated. Loss of only 30 per cent. of the moisture retarded growth and the growth rate appeared to be permanently reduced by this degree of desiccation. In atmospheres of equal drying power, plants of this phase appeared to lose water more slowly than younger plants. This, which may be related to cuticle development, offsets, to some extent, their greater susceptibility to loss of moisture.

The evidence from these experiments suggests that meristematic tissue is completely resistant to desiccation; both root and leaf primordia can be dried to the condition of the unimbibed embryo without injury. The increasing susceptibility of seedlings with age may thus be related to the increasing proportion of vacuolated to meristematic cells.

The failure of vacuolated cells to recover from a substantial loss of water is probably due to death of the protoplast following mechanical injury. The experiments of Iljin (1927, 1933, 1935) suggest that death of plant-cells on drying results entirely from mechanical rupture following rapid changes in volume. Adhesion of the protoplast to the cell-wall probably plays an important part. The entirely different behaviour of the cell-walls of root and of shoot following severe drying suggests that these tissues would provide valuable material for an extension of these investigations on the causes of death of cells by drying. Root tissue readily resumes its original configuration following immersion after drying, while shoot-cells remain wrinkled and folded. This may indicate an inherent difference in the properties of the cellulose in the two tissues, or it may result from a less rigid attachment of the protoplast to the cell-wall in the root than in the shoot.

The wheat seedling retains its drought-resistant properties up to a stage marked by the rapid extension of the first leaf. Susceptibility to drought then increases markedly, but capacity to recover from severe drying is not altogether lost. So long as some root primordia remain in a meristematic condition, eventual recovery of the plant from drought is still possible, for the leaf primordia can resume growth if functional roots are present to effect the necessary water uptake. The persistence of roots in the primordial condition during the early stages of growth is no doubt very important in the drought resistance of the seedling stage of cereals and grasses, and the normal development of the seminal roots in succession may be regarded as a characteristic affording protection against temporary water shortage.

SUMMARY

Seedlings of two varieties, one reputedly resistant and the other susceptible to drought, were desiccated to varying degrees at different ages, and their growth response on recovery studied. No varietal difference was recorded; both were highly resistant to drying and showed three distinct age-periods of varying reaction.

All germination stages from the dormant embryo until the coleoptile reached a length of 3–4 mm. were completely resistant to drought, as shown by elongation of severely dried plants proceeding on recovery at a rate comparable with undroughted plants. Until the emergence of the first leaf, plants were not permanently injured by a loss of 98 per cent. of the total moisture, although elongating roots were killed. At later stages less water was required to be lost before expanded tissue was killed and the growth rate was permanently reduced by a small loss of water.

These phases of differential drought resistance appeared to be related to the proportion of meristematic to elongated cells, the former apparently being completely resistant. The persistence of root primordia during the early growth stages would thus appear to be an important factor in the drought resistance of Graminaceous seedlings.

Some aspects of the behaviour of tissues during desiccation and recovery are discussed.

This work was carried out under the direction of Professor F. G. Gregory, F.R.S., to whom the writer is greatly indebted for suggesting the subject for the research and for his stimulating interest and criticism during its prosecution. The investigations were made while the writer held a Farrer Memorial Scholarship; to the Farrer Memorial Trust his most grateful thanks are extended.

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Quantitative Evolution

XVI. Increase of Species-number in Diatoms

BY

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With four Figures in the Text

GENERAL CONSIDERATIONS

THERE now seems to be a reasonable amount of agreement concerning many of the principles of quantitative evolution, but a brief statement of the present position may be useful for those who have not followed the recent developments in detail.

Duration. One of the main phenomena upon which attention has been directed is the occurrence of long durations for many species of both plants and animals. This point has been summarized by Mayr (1942, p. 244) thus: 'Fossil evidence indicates that the "life span" (the time between origin and extinction) of some species is very long; for others, it seems short. Few tabulations exist as yet of probable "life spans"'. One extensive tabulation of known 'life spans' for species of diatoms (Small, 1945*b*) and another of known 'stratigraphical ranges' for important genera of foraminifera (Glaessner, 1945) have since been published, while Cushman (1940 and 1948) gave generic geological ranges for all foraminifera, and Cushman and Ozawa (1930) gave species-durations for all the Polymorphinidae, a family of foraminifera.

These and other data support Mayr's statements (1942, p. 223) that 'speciation in marine animals moves at a snail's pace'; 'The paleontology of marine animals indicates the same slow evolution as does the existence of so many bipolar species'; 'The speed of evolution should not be over-rated, even in terrestrial groups. Many of the insects of the mid-tertiary Baltic amber cannot be separated specifically from living species.'

De Wildeman (1947) does not agree with the idea of long durations for some species, but he accepts quite willingly the idea of some extinctions being due to innate causes, such as spontaneous sterility.

So far as generic durations are concerned, it has long been recognized that many living genera had their origins in early Tertiary or even in pre-Tertiary times (Darrah, 1939; Simpson, 1944, p. 21). So far as species are concerned, the recognition that many living species have continued for many millions of years specifically unchanged is a relatively recent phase which contrasts markedly with the still-prevalent idea that all species are in a state of genetical and morphological flux. The evidence is mainly palaeontological and factual,

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and includes the above-mentioned amber insects, also molluscs (see Davies, 1934), foraminifera, and in considerable detail the diatoms.

Glaessner (1945, p. 6) considers that micropalaeontology 'appears to be about to take a leading part in the exploration of the organic world of the past', and (op. cit., p. 26) quotes Ellison on the conodonts thus: 'He came to the conclusion that, *as in many other groups of fossils*, most of the genera and species are long-ranging but that certain types (platform types) are valuable zone markers.'

Utilitarian palaeontology has been so concentrated upon 'zone markers' that the significance of long-ranging species has been largely neglected in evolutionary theorizing, although the 'father of stratigraphy', William Smith, found that some of the fossils in any particular bed might be the same as some of those from the beds above or below, while others might be peculiar to one bed (Holmes, 1944, p. 99).

Long-lived species of angiosperms are now being recognized, and a fossil forest of Upper Miocene age at Joursac in France, complete with fossil fungi, mosses, conifers, and angiosperm woody plants, was described by Hèribaud and Marty (1903). This included living species of birch, alder, hornbeam, hazel, beech, oak, willow, poplar, ash, walnut, &c. Modern techniques for microscopic identifications of leaves have established specific identities for Miocene fossil specimens of living species (cf. Axelrod, 1948, p. 133).

The more recent outlook upon these long durations for angiosperm species is to be found in papers by Chaney, Babcock, Stebbins, &c., and the general trend is for present geographical distribution to be used as suitable evidence for pre-Pleistocene distributions of the same species. Stebbins (1947) quotes a Miocene species, *Fothergilla Gardeni*, as living now in America; *Metasequoia* and *Latimeria* are other outstanding examples of ancient types for conifers and fish respectively; but the large Miocene specific element in our modern flora is only beginning to be recognized. 'In fact, we can safely make the generalization that within the Arcto-Tertiary forest the evolution of herbaceous elements of the flora has been as slow as that of woody species' (Stebbins, 1947, p. 152).

Babcock (1947, Pt. I, p. 138) has a section on the probable status of the genus *Crepis* in Miocene, in which he gives an early Miocene existence for at least twenty-one living primitive rhizomatous species in various parts of Asia, and a late Miocene existence for seventeen more living species in the same part of the world. In fact, once the slowness of speciation is recognized in terms of millions of years, the presence of ancient lineages, as species of long duration, becomes a necessity dictated by limitations of available time for most large genera. The old genera need not necessarily be large, as can be seen in diatom generic histories (Small, 1945*b*), a phenomenon which is recognized for angiosperms by Stebbins (1947, p. 156) where he states that 'groups with preadapted gene combinations, in order to undergo rapid evolution, must also be able to evolve reproductive isolating mechanisms. Some xerophytic groups of coastal California, like the genera *Yucca*, *Heteromeles*, *Adenostoma*, and

Erysimum, have undoubtedly become more abundant in individuals in recent times, but nevertheless have not produced many species.'

Zeuner (1946*a*, p. 377) makes the same point thus: 'The one fact that emerges with certainty from these comparisons of faunas is, that, in marine faunas, a large number of species and subspecies are stable, since they have not noticeably modified their characters since the late Tertiary.' He then gives evidence concerning Javan Miocene deposits, which suggests that 30 million years (30 m.y.) is a maximum duration for any one species; but some geological ranges of diatom species extend beyond 70 m.y. from upper Cretaceous to Recent, and a few foraminiferal species have had even longer life spans, such as from the top of lower Cretaceous to Recent or about 100 m.y., which is also the maximum species-duration indicated in studies of quantitative evolution among diatoms.

Speciation. Long species-duration is commonly taken as proof of no evolution or very slow evolution, but duration of a parental species is no measure of evolution when parental species are recognized as giving rise to other species by changes which affect only one or a few individuals while the parent species continues unchanged.

This results in monopodial evolution, or ancestral species coexisting with their derived species even into Recent times, as found for diatoms (Small, 1945–8) and as pictured for the genus *Crepis* (Babcock, 1947). The mechanism of this monopodial evolution is summarized by Babcock (op. cit., p. 145). The primary genetic processes causing evolution are there given as (1) gene mutations producing morphological and physiological differentiations and sterility barriers; and (2) some structural chromosome changes which act in 'the genesis of intraspecific sterility leading to discontinuity and hence to speciation'.

A combination of both these processes could occur in one or a few steps, and the second process is known to involve only one or a few generations; and since there is much evidence for the increase in numbers of species being very slow in time, the process of speciation must take place with long intervals of time between each rapid speciation. This rapidity of speciation as contrasted with very long intervals of specific stability has been discussed by Goldschmidt (1940), Simpson (1944), Just (1944), Zeuner (1946), Stebbins (1947), Rensch (1947), Small (1946, 1948), and others. General agreement seems to have been reached about the slowness of evolution combined with rapidity of changes when they do occur.

Stebbins (1947) suggests that there was an 'evolutionary burst' among angiosperms during Pleistocene 'involving species populations which came together after long periods of isolation in glacial refugia', and that this gave rise to the 'great predominance of polyploids in the glaciated areas' by hybridization and chromosome doubling, allopolyploidy, &c. (cf. Löve and Löve, 1948). Another similar 'burst' is said to be just beginning as the result of hybridizations resulting from the colonization of new habitats being created by human activities of various kinds. Just (1947) summarizes many opinions about 'bursts of production' followed by 'periods of relative stability' particularly among angiosperms.

There need not be any significant parallel developments, comparable with all this Tertiary and post-Tertiary speciation on land surfaces, among the numbers of species of marine micro-floras or micro-faunas, such as diatoms and foraminifera.

Quantitative analyses. Evolution, or change in time, has been slow, and the process of speciation has been relatively rapid. Therefore some actual historical data are very necessary for further progress in our understanding of these processes. The present numbers of species in various taxonomic categories, such as genera, families, or larger groups of species, must have been attained by some arithmetical process or processes. There have been additions and subtractions, as origins and extinctions. On the old views of evolution, which may be described briefly as sympodial, each addition of one or two new species involved the subtraction or extinction of the parent species; but with the new view, of coexistence and monopodial developments, each addition does not necessarily involve a simultaneous subtraction, so that subtractions or extinctions cease to have the direct correlation with origins, which was assumed by Simpson (1944, pp. 17, 20–7, 128, 131–3), following the old general view that one organismal form disappeared because it *changed into* another form. Simpson, however, found that the data for generic durations ('survivorship') in Pelecypoda (bivalve molluscs) demonstrated the presence in his records of two quite distinct types of duration (op. cit., p. 133). The only logical way of investigating the frequency distributions of generic and specific durations is to measure and count such data in a group for which the records are as nearly complete as possible with our present knowledge. This has been done for diatoms (Small, 1945–8) and for the genera of Foraminifera (Small, 1948*b*).

History of present numbers. There have been various theories to account for the present numbers of species and genera. According to the views held by Linnaeus they were all or nearly all attained by a direct process of special creation, and this involved no objective arithmetical development. According to the Darwinian and neo-Darwinian views these present numbers are the result of multifarious causes which have not been separately analysed to any considerable extent and which have therefore given results that may be taken as governed by the laws of probability. The frequency distribution of such numbers should follow various probability curves, and the multifarious causes are regarded as so complex that they cannot all be analysed out arithmetically or quantitatively in any other way.

Sources of data. Arithmetical analyses require some kind of definite arithmetical data, and it is now generally recognized among those who use such data that the most suitable and comparable lists of species and genera are those compiled by one person in a review of one large group. Mills's 'Index to the Genera and Species of the Diatomaceae' (1933–5) has been taken as a fixed book of reference for numbers of valid species and genera of diatoms.

These numbers have been used throughout for arithmetical analyses, but they are not completely definitive, although they are more or less precise and refer to a definite stage in our knowledge of the diatoms. They represent something over 90 per cent. of a complete diatom history, with about 7,000 species listed, and something less than 700 more described since 1935 or likely to be described later. The number of new *fossil* species described for Tertiary deposits is relatively small, but there has been a large increase (about 107 in known upper Cretaceous diatoms (Long, Fuge, and Smith, 1946), and also an extension into Cretaceous of the stratigraphical ranges of 43 species already known in Tertiary deposits. The new fossil species have been described by Hustedt (1935–45), Iyengar and Subrahmanyan (1943), Reinhold (1931–7), Silva (1946), Subrahmanyan (1946), and others.

Fossil diatoms. There is a very large literature dealing with Tertiary deposits of the siliceous shells of diatom frustules. The microscopic complexities of structure make it relatively easy to establish the specific identities of these well-preserved fossils, and new species have been sought by diatomists for over a century. The resulting knowledge is given in a multitude of records which are listed in the bibliography given in Mills's 'Index'. For five years most of my time was occupied with a routine tabulation of geological records for all fossil species of diatoms. By covering all the available literature, and particularly by obtaining much help from the diatomists, F. W. Mills, Esq., and Dr. D. McCall, all the species admitted as valid in Mills's 'Index' were placed in geological time. The main sources of information have been enumerated elsewhere, with the tabulated results (Small, 1945*b*).

Time-scale and deposits. The chronology used includes an initial Cretaceous (C) for relatively few species, followed by Paleocene (Pa); lower, middle, and upper Eocene (LE, ME, UE); Oligocene (O or Og); lower, middle, and upper Miocene (LM, MM, UM); lower and upper Pliocene (LP, UP); with Pleistocene and Holocene taken together as Recent (R). This divides the approximate 70 million years of Tertiary into ten approximately equal subperiods, with a tendency for the Neogene five to be rather shorter than the Paleogene five subperiods (Holmes, 1947, p. 145). For the calculations used here in Tables I and II, and in Figs. 1–4, two additional subperiods of equal length have been introduced for upper Cretaceous, as UC3 and UC4.

The various diatom deposits were placed in these subperiods by following accepted geological opinions, and by careful analyses of Hèribaud's records. The chronology used for named deposits is given in detail as Table III in Small, 1945*b*.

The diatom diagram. From all this routine tabulation of geological records for species of diatoms, very little of interest emerged until it became obvious that species can be classified into two categories with geological ranges or durations which have been either '*short*' confined mainly within part of one subperiod and not extending beyond two adjacent subperiods, or '*long*' mainly from the species origin up to Recent and always covering more than two subperiods. When all the species with Tertiary fossil records were thus classified into evanescent or *e*-species, and permanent or *p*-species which have persisted or tended to go on in time, a large number of interrelated regularities were soon uncovered within the mass of tabulated durations. In fact, it became clear that diatom history was a rather complicated diagram of numerical evolution in a group which was free from most of the disturbing factors of life on a land surface.

These factors include scarcity of inorganic food and for animals organic food, restriction of suitable habitats or ecological niches, glaciation, frosts, droughts, and obligate bisexual reproduction for higher animals as contrasted with the extensive asexual multiplication of diatoms.

Most diatoms are marine, living in large ocean currents as unicellular autotrophic organisms multiplying by vegetative cell-divisions and only occasionally producing reproductive auxospores, by apogamy or autogamy in centric types and some pennate types (Fragilariaceae), and by the allogamous fusion of nuclei from two parental cells in the other pennate families, here distinguished as the allogamous Pennales. There are also many freshwater species, mostly pennate types; and some pennate types which have a raphe and are mobile can exist in moist soil, often as much smaller organisms than their typical aquatic forms (Lund, 1945–6).

The details of diatom history show that centric types were the usual diatoms during early Tertiary, and that there was an increasing predominance of pennate types in late Tertiary, but even now most Centrales are marine diatoms and most freshwater diatoms and all soil diatoms are in Pennales.

The regularities of arithmetical developments in numbers and durations of diatom species have been expounded and summarized in various ways (Small, 1945–8), but this contribution deals with a further analysis of the factors which have contributed to the observed increase of species number in the two main groups, allogamous Pennales and Centrales.

The allogamous Pennales exemplify a relatively young group in which the *initial* phase and the *increase* phase can be very clearly traced and analysed quantitatively, and for which there is only doubtful evidence of an obscure beginning of the phase of *decline*, which is usually part of the detailed numerical structure during the apparently *stationary* phase.

The Centrales, on the other hand, constitute an example of a relatively old group which is passing from the phase of *increase* towards a *stationary* phase within which quite considerable elements of decline can be traced in the histories of single genera.

There is, therefore, some interest in analysing the numerical factors which are similar in type but different in arithmetical values for these two groups. This is no longer an isolated arithmetical curiosity: these analyses show how the various phases in the growth, development, and decline of various taxonomic units can be understood as the result of measurable durations of units and rates of production of units, which are more or less regular in time and which show some irregularities as modifications introduced by external circumstances.

THE ALLOGAMOUS PENNALES

As one very clear example of an *initial* phase followed by a phase of *increase* with multiplication factors for temporary and residual increase of numbers, the history of species-number in all the allogamous families of Pennales taken as one group is very instructive.

Present total. The total number of species in this group, allogamous Pennales, is 3,526 for species occurring in Recent, including Pleistocene. This total refers to species accepted as valid in Mills's 'Index' (1933–5). Several hundreds of new species have since been described, mainly in Recent habitats, so there is no use in laying stress upon the precise number except for arithmetical purposes; see Hendey (1937), Hustedt (1935–45), Ross (1947), &c.

The only species of this group which are recorded for Paleocene are three in the genus *Navicula*, *N. praetexta*, *N. hennedyi*, *N. yarrensis*; all three are known to be now living, and the first two are known from very many deposits throughout Tertiary.

From these initial three species the present total of 3,526 could have been attained in various ways, but the factual history shows an approach to a more or less regular geometric progression, which can be expressed as: final number $= i \times 2^n$, where i is an initial number and n the number of geological subperiods, taken as approximate tenths of the total for Tertiary time (about 70 million years). There are, of course, many other ways of expressing this relation, see Small (1945–9) and examples of normal statistics. The subperiod time scale is only approximately uniform, so that the intermediate values for species-numbers in successive subperiods can be only roughly approximate to any uniform series, even if the actual rate has been completely constant in relation to uniform time.

These approximations are sufficiently precise and regular to show some arithmetical detail, including a distinct and measurable difference between the temporary increases within each subperiod and the overall or residual increases which have contributed to the building up of the present or final total.

Mathematically, the number 3,526 is equal to (*a*) $0{\cdot}8608 \times 2^{12}$, and is also equal to (*b*) $1{\cdot}0000 \times 2^{11{\cdot}7835}$, and both these series approximate to (*c*) $3{,}526 \times 0{\cdot}5^{12}$. Taken to the nearest unit these series are:

(*a*) 1–2 — 3–7 — 14–28 — 55–110 — 220–440 — 881–1763 — 3526;
(*b*) 1–2 — 4–8 — 16–32 — 64–128 — 256–512 — 1024–2048 — 3526;
(*c*) 1–2 — 4–7 — 13–27 — 55–110 — 220–440 — 881–1763 — 3526;
(*d*) (1–2)— 3–3 — 8–20 — 64–185 — 296–316 — 575–443 — 3526.

The fourth series (*d*) consists of the known total numbers of species occurring in each of the subperiods of Tertiary to Recent for allogamous Pennales. This factual series (*d*) fits reasonably well to series (*a*) or (*c*) which are exactly the same as each other except that of '3 or 4' the latter was taken for (*c*) and also 27 for 28 and 13 for 14 (Fig. 1).

Apart from the general approximate fitting, the first two steps are still hypothetical, in brackets, and there is a definite shortage in the known number

(443) for upper Pliocene, and possibly for lower Pliocene. In all diatom records there is this Pliocene gap, because the marine deposits of diatoms for upper Pliocene and in part for lower Pliocene are still unknown since they lie below the Recent deposits at the bottom of modern seas.

Residual increase analysed. Since some of the new species produced in each subperiod are not known outside their subperiod of origin, these species cannot have given rise to the other species which arose in subsequent subperiods. Only those species which continued into subsequent subperiods could be possible parents. In general, only the permanents or *p*-species could possibly have been parental. (*Note*: 'permanent' = tending to go on in time, used of form or things; while 'persistent' = tending to continue in the same course, used mainly of persons, without particular reference to time, except for its technical use in botany.)

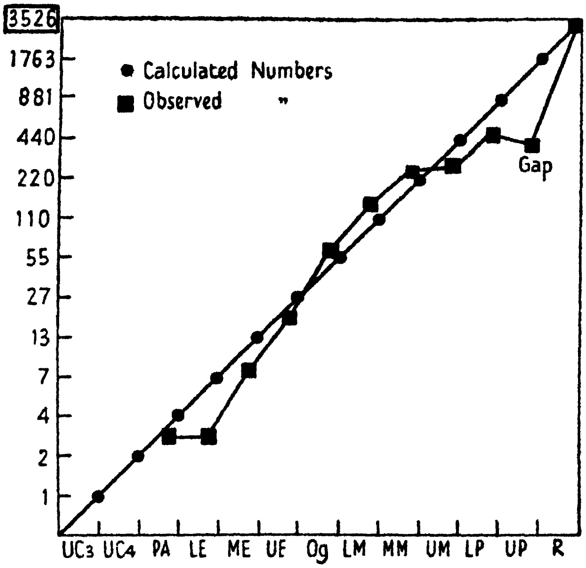

FIG. 1. The total number of species in the allogamous Pennales, occurring in different geological periods (for symbols see p. 95). The calculated numbers assume that the order of increase is regular as given by 1×2^n, where *n* is the number of periods. The vertical scale is this geometric series.

The residual or overall increases clearly depend upon the numbers of old *p*-species which, in each subperiod, have come on from previous subperiods. The rates of *temporary* subperiod increases can be measured as new/old or *A/O*, as for production rates in any taxonomic categories (Small, 1948*b*), using the numbers of new species and numbers of old *p*-species known in any given subperiod.

For allogamous Pennales the values for *A/O* during Tertiary have been as follows, starting with an initial lag of two zeros for no observed increase in Paleocene and lower Eocene: 0, 0, 1·0, 1·06, 4·31, 2·38, 1·47, 1·45, 1·39, 0·27, 6·13; which averages out at 1·77 (Small, 1948*a*, p. 300). This 1·77 is the average ***temporary increase*** per subperiod, therefore the total including the original parents would be 1+1·77 or $\times 2{\cdot}77$ for each average subperiod. But this rate of increase, if continued even only from middle Eocene, would have given, from the original 3 species, 7,820 in upper Pliocene and 21,660 in Recent, so that some reduction in the rate of increase must have occurred to give an approximation to $\times 2{\cdot}0$ per subperiod for the observed increase.

The reduction of the observed temporary to observed residual increase might have been applied irregularly or regularly, by intermittent cataclysms as suggested by Yule (1924) or by extinctions due to less regular catastrophes: but the observed residual increases have been more or less regular as can be seen by their fitting to a $\times 2^n$ diagonal in Fig. 1 and by comparing the (*a*) or (*c*) series with the (*d*) series given above. Increase has, historically, proceeded as 1·7 steps forward (temporary increase) followed by 0·7 steps backward with the disappearance of short-lived species in each subperiod, leaving a residue of 1·0 steps forward as the new *p*-species continued (residual increase).

The numerical data for Fig. 1 are given in Table I. The ratio of 9 old *p*: 9 new *p*: 7 new *e*, as the composition of the total species in any one subperiod, means that there will be 18 *p* to 7 *e* for each 25 species occurring. On this basis there should be $\frac{18}{25}$ths of 3,526 (or 2,540) *p*-species among the total occurring for Recent in allogamous Pennales: and since new *p*-species have arisen from old *p*-species in the proportion of 9 new from 9 old, the total number of *p*-species can be calculated for each Tertiary subperiod as a decreasing series from $2{,}540 \times 0{\cdot}5^n$. This series (*a*) and the numbers of *p*-species known for the corresponding subperiods (*b*) run as follows:

(*a*) 2,540 — 1,270 — 635 — 317 — 158 — 79 — 40 — 20 — 10 — 5 — 3 — 2 — 1
(*b*) (441) — (442) — 445 — 280 — 196 — 134 — 36 — 15 — 7 — 3 — 3 — ? — ?

Because *p*-species are defined by their geological ranges, the first two items include only old *p*-species; then 445 for lower Pliocene and earlier subperiods fit reasonably well, on the whole, down to the original three species of *Navicula* in Paleocene. The fitting shows quite clearly in Fig. 2, where the diagonal is the series (*a*) above (final number $\times 0{\cdot}5^n$), and the line with black squares is the observed totals for *p*-species in each subperiod, series (*b*) above.

The totals for *p*-species can be broken up into two halves corresponding to the 9 old: 9 new. The *e*-species also can be analysed out as $\frac{7}{25}$ths of each subperiod total, and this series also is, mathematically, a decreasing $\times 0{\cdot}5^n$ series; but, again by definition, the *e*-species among the Recent total cannot be distinguished, and again there is the Pliocene gap in our knowledge particularly of *e*-species for upper Pliocene. These three series of calculated and observed numbers do not fit so closely as those in Figs. 1 and 2, but they are set out with the others in Table I for reference.

Thus, with a subperiod production-rate averaged at 1·77 for temporary increases in numbers of species, and with an *e*/*p* ratio of 7/9 for reduction of these temporary increases by $\frac{7}{16}$ths as the proportion of species which have

TABLE I

Data for Allogamous Pennales

Geological period	Calculated species-number*					Observed species-numbers				
	Total *OC.*	Total *p*18:	Old *p*9:	New *p*9:	New *e*7	Total *OC.*	Total *p*	Old *p*	New *p*	New *e*
Recent	**3,526**	2,540	1,270	1,270	986	**3,526**	441	441	—	—
Upper Pliocene	1,763	1,270	635	635	493	443	442	437	5	1
Lower ,,	881	635	318	317	246	575	445	280	165	130
Upper Miocene	440	317	158	159	123	316	280	191	89	36
Middle ,,	220	158	79	79	62	296	196	134	62	100
Lower ,,	110	79	40	39	31	185	134	36	98	51
Oligocene	55	40	20	*20	15	64	36	15	*21	28
Upper Eocene	27	20	10	10	7	20	15	7	8	5
Middle ,,	13	10	5	5	3	8	7	3	4	1
Lower ,,	7	5	3	2	2	3	3	3	—	—
Paleocene ,,	4	3	2	1	1	3	3	3	—	—
Upper Cretaceous 4	2	2	1	1	—	—	—	—	—	—
,, ,, 3	1	1	—	1	—	—	—	—	—	—

* Calculated from total *OC.* observed 3,526 R, assuming regularity of the ratios given at the head of each column.

not continued beyond their subperiod of origin, there has been a clear approximation to a $\times 2^n$ residual increase which, arithmetically, is the result of the arithmetical processes summarized as:

$$9 \text{ old} + (16 \text{ new} - 7\ e) = 9 + 9 \text{ or}$$
$$\times[1 + (16/9 \times 9/16)] = \times 2 \text{ per subperiod,}$$

as the common ratio of the geometric progression occurring in the residual increase of species-number in allogamous Pennales.

The additions of new species, and the subtractions of evanescent *e*-species, and the consequential geometric progression or multiplication of permanent species, have all been approximately regular throughout uniform time. This, as has been pointed out, refers to a group in its *phase of increase* during which the number of new species arising is a function of the number of old species continuing in the living condition as possible parent-species.

Rate of increase. The rate of increase in numbers of species among the allogamous Pennales is higher than that for Centrales, but it is a very low rate. The ratio of 16 new from 9 old species per subperiod of about 7 million years means an average increase of one new from one old species in $\frac{9}{16}$ths of 7 m.y. or about 4 million years. Since gradual genetic changes of populations would have made chaos of the diatom fossil record, the actual process of change must have been very rapid with intervals of complete fixity for about 4 million years between each rapid change in any one lineage. These rapid changes left the parent species to continue unchanged, so the change could have affected only a part of the current population.

The simplest way to meet *all* the known facts is to regard each speciation as a one-step change in one organism of the parent species. Mayr (*in litt.*)

agrees that 'asexual reproduction is conducive to evolution by sudden steps, instantaneous speciation', and there is abundant asexual multiplication of unit frustules among all diatoms, which could preserve purity of the new lineage after any specific mutation.

The allogamous Pennales is an example of how the arithmetic of evolution, as change in the numbers of species, has in fact proceeded during the *initial* or lag phase and the *increase phase* of group development. There is some, but very little, evidence in generic details that this group is now approaching its stationary phase after a period of increase which began in upper Eocene about 50 million years ago, and this would agree with Zeuner's views about the length of the 'explosive' phase of evolution within a group of any taxonomic category (Zeuner, 1946*b*, p. 248; see also below).

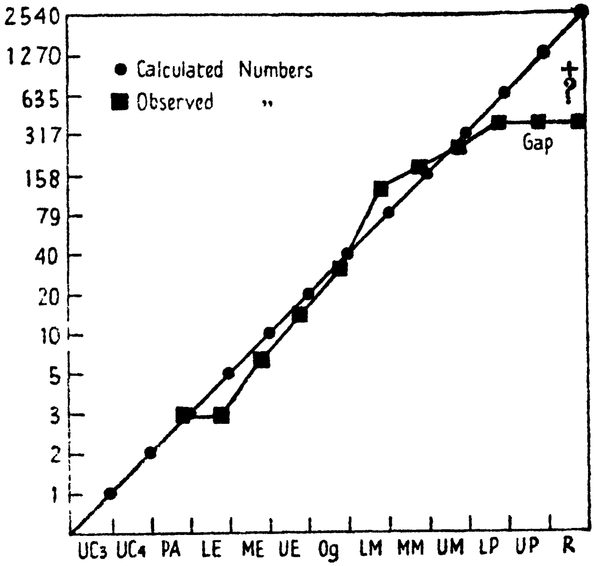

FIG. 2. The total numbers of permanent or *p*-species in allogamous Pennales at different geological periods (see p. 95 for symbols used). The vertical scale is based on the geometric series 1×2^n, where n is the number of periods.

In order to appreciate the significance of this analysis it should be noted that the component factors have been 'observed' separately in the detailed data, and the more or less constant value of the resultant factor is derived by simple multiplication and the addition of 1·0 as the parental proportion of the total number occurring; thus, $1+16/9$ for temporary increases, and $16/9 \times 9/16$ for proportion of *p*-species continuing.

The fitting of the factual observed numbers to the calculated diagonals, in Figs. 1 and 2, leaves little doubt that the main factors have been analysed out. These are (1) a more or less constant proportion of new species, the number of new species having been controlled by the number of old *species* available as parents and consequently not by any other characteristic such as number of individuals per species or number of generations per species, or variations in external factors; and (2) a more or less constant proportion of species which have continued into subsequent subperiods, the actual numbers of such species having been controlled by the total numbers of species produced (*a*) in each new subperiod *quota* and (*b*) in every previous subperiod quota.

The large degree of regularity observed in the functioning of these characteristics, during the Tertiary history of allogamous Pennales, is a demonstration that these characteristics are innate genetic factors which have functioned undisturbed, or at least with comparatively little interference from factors outside the organisms concerned. Both species-durations and the distribution of duration types have been inherited characteristics, and so also has been the degree of specific mutability as measured by the subperiod speciation rate.

Further implications. Zeuner (1946*a*, chap. xii; 1946*b*) in his discussions of biological evolution in relation to time, has assembled many examples of various numerical phenomena, such as absolute ages of various groups of diverse taxonomic categories; these ages in millions of years (m.y.) vary from 1 m.y. to 500 m.y., mostly according to the origins rather than completed durations because nearly all the groups given by him have continued into Recent. Zeuner (loc. cit.) also gives many examples of numerical histories in which there have been (1) a relatively insignificant *initial or lag phase*, (2) an *explosive phase* of rapid increase 'which is suggestive of a logarithmic increase' (op. cit., p. 356), (3) a climax or *stationary phase*, and (4) a '*phase of decline* which may be sudden or slow'.

The first two phases indicate 'an underlying exponential function or an increase per unit which is determined by a certain constant factor of multiplication', as Zeuner puts it (op. cit., pp. 357–8). This, it should be noted, introduces an element of multiplication into the arithmetical history, and also the extra idea that the number of new units has been determined by the number of old units. The significance of this arithmetical phenomenon during the phase of increase is fundamental, as it means there is no 1 : 1 correlation of origins and extinctions.

The arithmetical value of the multiplication factor varies from period to period within relatively narrow limits in some cases. Zeuner has expounded the 'explosive' phase of increase in tables and in time-frequency diagrams for species in the genus *Salenia* (a sea-urchin), for species in the family Clypeastridae (sea-urchins), and for genera in the family Spiriferidae and the superfamily Spiriferacea (Brachiopoda). He gives total numbers of units as linear magnitudes, and also in histograms as block-areas against uniform basal time-scales, so that curve-forms rather than actual data are presented.

Small (1948*a*) has given for diatoms the actual arithmetical data grouped in various ways and plotted against an approximately uniform time-scale.

New units cannot arise from old units which are extinct at the time of the origins of these new units, so the old units which are not extinct at the time of origin of new units are the only possible parents for new units in whatever taxonomic category the units are taken. Then the number of new units arising (A) is proportional to the number of persisting old units (O) in a ratio which is measurable as A/O; this gives a measure of the rate of production of new units for any given section of time. This ratio, as $1+A/O$, is a measure of the multiplication factor or the rate of increase from point to point along a uniform time-scale for the whole duration of groups of units. It has been so used for speciation-rates of the genera and of the main groups of diatoms (Small, 1946 and 1948*a*), also for the production-rates of families and genera in foraminifera, of genera and species in Polymorphinidae (foraminifera), also genera and species in the two main groups of diatoms (Small, 1948*b*).

Among taxonomic units of upper Cretaceous or Tertiary origin, the first two phases, *initial* and *increase*, may be the only phases present in the records; but among units with earlier origins the data may include the third or *stationary phase* and possibly also the fourth or phase of *decline*. It is now possible to analyse out various factors which contribute to the production of these various phases, particularly in the history of the diatoms.

According to Zeuner (1946*b*, p. 248) the phase of *increase* or 'explosive phase' has commonly lasted for about 50 million years, and this length has been quite independent of the taxonomic category or kind of organisms considered. It has been of the same order of duration for species, genera, and orders: also, 'The significance of this apparent limitation of the duration of explosive phase is still obscure.' He suggests that it may mean that 'the number of species-steps involved in the evolution of a new family or order is not greater than that involved in the evolution of new genera'. Small (1948*b*) has shown that the origin of all new units of whatever taxonomic category can be traced to one new change which may be one of various qualities, the quality of the change determining the taxonomic category of the new specific unit, as suggested by Sewertzoff and by Goldschmidt (1940) (see Zeuner, loc. cit.).

THE CENTRALES

As an old group, with a few of its earlier large genera now much reduced in living species and a few now large genera which have produced only a few *p*-species since middle Miocene, the Centrales shows signs of the action of the same component factors as have been distinguished in the data concerning the allogamous Pennales. Nevertheless there are various large differences due to relative age, to the presence of an element of decline, and of different arithmetical values for the two main factors of overall increase.

Present total. The total number of species in this group, Centrales, is 1,438 for species occurring in Recent, including Pleistocene. This total refers to species in Mills's 'Index' (1933–5) and, as mentioned earlier, only a few dozen new species have since been described for Tertiary and Recent, although 107 new species have been described from the pre-Tertiary Cretaceous deposits at Moreno Gulch.

In the case of the allogamous Pennales the *initial* or *lag phase* is present in the Tertiary history of the group, as the initial 3 *p*-species for Paleocene and lower Eocene, but with the Centrales there was at least one large generic complex, *Hemiaulus-Trinacria*, which had reached its maximum in or before Paleocene, so that here we are dealing with an old group which had probably a long pre-Tertiary history before the arithmetical phenomena of its Tertiary history were developed.

Overall increase analysed. In Centrales the proportion of evanescent or *e*-species in the Tertiary subperiod quotas of new species has been demonstrated as having been 3 *e*-species to 1 *p*-species. This 3/1 *e/p* ratio has been proved as having been maintained throughout the Tertiary subperiods for Centrales as a group, for many centric genera each as a whole, and in generic subperiod quotas of new species (Small, 1945*a*, 1946, and 1948*a*, pp. 265–75).

This large proportion of *e*-species, three-quarters of the total of each subperiod quota, makes it rather more difficult to use the total number of species occurring in any one subperiod as a suitable basis for detecting any underlying regularities in the component factors of increase. The Pliocene gap is almost certainly larger for centric species than for pennate species, because more species of Centrales are exclusively marine. Some of the pre-Tertiary fossil *e*-species are probably mixed up with the Paleocene records, many of which are from submarine deposits near Franz Josef's Land. The Moreno Gulch Cretaceous deposits were at first said to resemble closely some of the Russian deposits which have been taken as Paleocene (Hanna, 1927; Small, 1945*b*, p. 297), so the records for the Russian deposits, especially the submarine ones, may well include a fossilized accumulation of *e*-species from pre-Tertiary times. There are also old submarine deposits of diatoms along the west coast of Africa, between Dakar and Walfish Bay, which very probably include many pre-Tertiary species, but the analysis of Leuduger-Fortmorel's records (1898) has been a long and complex piece of work quite beyond the scope of this present contribution.

With all these irregularities and uncertainties the *e/p* ratio of 3:1 stands out as clearly demonstrated in considerable detail (Small, 1948*a*, pp. 265–75). This was the first regularity to be uncovered among the diatom data (Small, 1945*a*) and was the key to all the other regularities. The maintenance of this ratio of 3 short-lived or *e*-species to 1 long-lived or *p*-species in most of the centric genera, in all the subperiod quotas and in the group as a whole (1,230:403), made various other analyses possible.

The analysis of subperiod speciation rates in allogamous Pennales was carried out above by averaging the *group* rates, but in the case of Centrales there are several factors, such as decay of speciation in some genera, which have led to a better analysis by other methods of approach.

Each one of the 351 generic subperiod speciation rates has been set out for all three main groups of diatoms (Small, 1948*a*, p. 305), and, when those for Centrales are examined and grouped (op. cit., p. 307), it is found that the distribution of these rates is such that there are 43 values up to 0·45, 80 between 0·46 and 1·82, with 32 at 1·0, and 60 above 1·83. Since there has been a mode somewhere around 1·0, and since this subperiod speciation rate yields the best fitting of detailed facts to *generalized* residual increase data, the analysis of these data has been based upon this value.

The *temporary* subperiod increases can be measured as A/O or new/old species in each subperiod. They have been, starting with lower Eocene, as follows: 1·12, 0·8, 3·68, 1·13, 1·48, 0·97, 0·52, 1·14, 0·14, 3·77; which averages out at 1·475 per subperiod for Centrales, instead of 1·77 as for allogamous Pennales (Small, 1948*a*, p. 300). In all subperiods, except middle Eocene, the pennate subperiod production rates for species have been higher than the corresponding centric production rates to the extent of $\times(2{\cdot}0 \pm 0{\cdot}8)$, but since these are logarithmic characteristics the consequential values do not have straight arithmetical relations. The extreme values for subperiod production rates in the two groups AP/C are 0·27/0·14 and 6·13/3·77, and these yield ratios of 1·9 and 1·6, average 1·75, which, with the allogamous Pennales increase being about 1·77 per subperiod, again yields about 1·0 for the corresponding temporary subperiod increase in Centrales (Small, 1948*a*, p. 300).

The average value of 1·475 for *group* production rates is too high to fit the residual increase data, but if the modal value of 1·0 for generic subperiod production rates is taken as a basis, this can be combined with the known proportion of *p*-species (1/4). Adding the parental 1·0, the answer to this sum is: $1+(1\times 1/4) = 1{\cdot}25$, as a numerical value for the residual increase of species number in Centrales, as compared with $1+(16/9\times 9/16) = 2{\cdot}0$ for allogamous Pennales.

This 1·25 is the common ratio (r) of a rather slowly rising geometric progression: it gives the 'slope' for rectilinear diagonals corresponding to those for allogamous Pennales in Figs. 1 and 2, where r is 2·0. But the problem of fitting involves choosing a suitable starting-point. For the allogamous Pennales the value of 1 could have been taken, but the final (present) value of 3,526 was in fact taken and divided by 2·0 to give a reducing series which, of course, is much the same as starting with a hypothetical 1, and applying the lowest known number of 3 to the corresponding point on the mathematical diagonal.

In the case of Centrales, the suitable known number could not be anything like 3, because of the long pre-Tertiary history of this group, and the earliest total, that for Paleocene, is suspected to contain a pre-Tertiary element of *e*-species. The Paleocene *p*-species are not affected by this uncertainty because their subsequent Tertiary history proves them as permanent, and whether they began in Cretaceous or in Paleocene does not affect their status as valid *p*-species known in Paleocene. The number of *p*-species in Paleocene (91) was, therefore, taken as a suitable point for the application of the observed data to the diagonal calculated for *p*-species.

With this point of application settled, the calculated distribution of *p*-species throughout Tertiary becomes a regular geometric progression in which each successive term is $(\times 1{\cdot}25)^n$, following the formula for residual increase $1+(1\times 0{\cdot}25)$ but using only *p*-species, and starting with 91 in

Paleocene as equal to $\times(1+0{\cdot}25)$, giving 73 old and 18 new *p*-species. This series, from Paleocene onwards, for total *p*-species in the succession of subperiods (*a*) can be compared to the observed series for the same subperiods (*b*) as follows:

(*a*) 91 — 114 — 142 — 177 — 221 — 276 — 345 — 431 — 539 — 674 — 842;
(*b*) 91 — 105 — 119 — 203 — 228 — 273 — 309 — 269 — 280 —(259)—(259).

By the definition of *p*-species, at least the last two items of series (*b*) cannot be fitted because the necessary durations are not known for species which

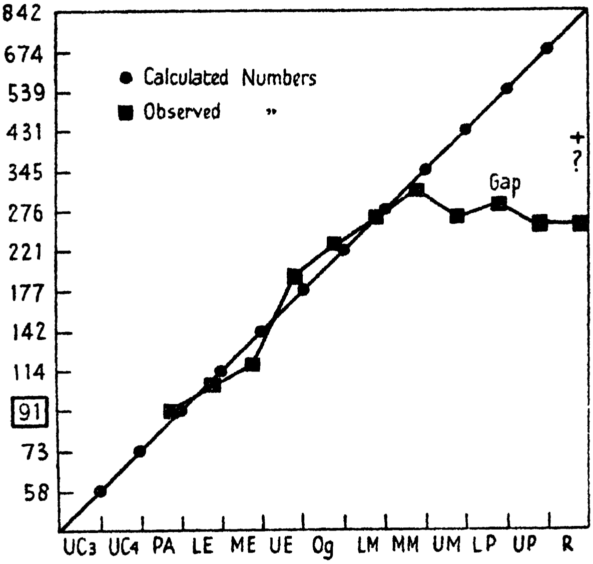

FIG. 3. Total permanent or *p*-species of Centrales in different geological periods (for symbols see p. 95). The vertical scale is a geometric series described in the text.

began in upper Pliocene and Recent. The same might be said to apply to lower Pliocene, but there is also a deficiency of species with LP-R and UM-R records. This is a real phenomenon in the records, which may be in part an extension of the Pliocene gap in these records for mainly marine genera: it is also due in part to a decrease in subperiod speciation or to a smaller than normal increase in subperiod speciation among genera which ceased to expand in or before upper Miocene. The detailed facts can be followed in the basic tables of generic histories (Small, 1945*b*, Tables I and II).

Starting with 91 *p*-species in Paleocene, using the factor $\times 1{\cdot}25$ per subperiod for *p*-species only, the (*a*) and (*b*) series for calculated and observed numbers can be graphed as in Fig. 3, where the fit of the observed numbers is seen to be reasonably good from Paleocene to middle Miocene, after which the decline in production of *p*-species in most large genera combines with the deficiency due to the definition of *p*-species in the appearance of a gap between observed and calculated numbers. This 'gap' thus has a double origin, (1) the impossibility of assessing the duration from UP to R or in Recent-only as 'long', and (2) a recognizable decrease in *p*-species production by many genera for lower Pliocene and upper Miocene. The second factor is a recognizable element of the *decline phase*, and another aspect of the same decline is to be traced in the extinctions during middle Miocene of species which have been long-lived: there were 46 of these extinctions in MM, 17 in UM, and 23 in LP (Small, 1946, fig. 4, p. 61). But sufficient long-lived species continued, in the now large genera, to give from their accumulated numbers the known larger total numbers of species found only in the Recent.

There has been as yet no similar decline in allogamous Pennales, where only 5 genera and 14 *p*-species have become extinct, as compared with 61 genera and 144 *p*-species now extinct in Centrales (Small, 1946, figs. 2 and 4).

Centrales subperiod totals. Bearing in mind the various degrees to which old genera in Centrales have passed into generic *phases of decline* especially in production of new *p*-species in late Neogene (UM to UP), and also the generic maxima for *e*-species shown by some large genera for Paleocene, upper Eocene, and middle Miocene, no great regularity can be expected within the relatively small residual increase for *total number* of species, which has occurred over the ten subperiods of Tertiary time and into Recent. But these observed numbers can be compared as a series (*b*) with a calculated series based first upon an increase of *p*-species from 91 in Paleocene by × 1·25 per subperiod. It is necessary next to add $\frac{3}{5}$ths of the number so obtained to each subperiod quota as the proportion of *e*-species which would be given by the distribution of each subperiod quota in the proportion of 4 old *p*-species to 1 new *p*-species and 3 new *e*-species. This 4 old *p*: 1 new *p*: 3 new *e* for Centrales contrasts with 9 old *p*: 9 new *p*: 7 new *e*-species for allogamous Pennales. The distribution of new species as 1 new *p*: 3 new *e* is in accordance with the *e*/*p* ratio of 3/1 which has been observed for subperiod quotas of new species in the Centrales as a whole (Small, 1945–8).

These two series of numbers, (*a*) calculated and (*b*) observed, for the total numbers of Centrales in the subperiods Paleocene to Recent are:

(*a*) 146 — 182 — 228 — 284 — 354 — 442 — 552 — 690 — 862 — 1,078 — 1,348;
(*b*) 361 — 132 — 127 — 356 — 372 — 426 — 523 — 299 — 359 — 260 — 1,440.

The fit, from the second item (lower Eocene) to the seventh item (middle Miocene), is better than might have been expected. The deficiency in middle Eocene, 127 for 228 calculated, is certainly due to our lack of knowledge since the *e*-species for that subperiod are known only from the Kreyenhagen shales (Hanna, 1931), assuming these do belong to that subperiod. The 'excess' for Paleocene, 361 for 146 calculated, almost certainly includes a large admixture of pre-Tertiary *e*-species from submarine deposits allocated to Paleocene, and quite possibly other Cretaceous *e*-species from the lower parts of the Russian deposits, which have been classed as Paleocene but closely resemble the material from Moreno Gulch, which is now very definitely placed at the top of Upper Cretaceous (Hanna, 1927; Long, Fuge, and Smith, 1946). The point for 361 in Paleocene is marked and isolated in Fig. 4, where the above two series (*a*) and (*b*) are graphed against the usual subperiod time-scale.

The large difference, between the upper Eocene with its included generic maxima and the middle Eocene (ME) which is known to be minimal in our knowledge of its *e*-species, lies across the diagonal, but a direct connexion of the LE point to the UE point would present a reasonable fitting. It must be remembered that these numbers differ very little, as compared with the $\times 2^n$ progressions in Figs. 1 and 2, and also that here we are dealing with a very mixed collection of genera in different *phases* of generic development. The extensive gap from upper Miocene (UM) to upper Pliocene (UP) corresponds closely with the similar gap for *p*-species, which has already been discussed above and illustrated in Fig. 3. The problems raised by these divergences are slightly different, but the deficiencies again have a double origin: (*a*) there is still the upper Pliocene gap in our knowledge, particularly of marine species which began and ended in that subperiod, that is, of upper Pliocene *e*-species which lie below the Recent deposits of diatoms at the bottom of existing oceans; and (*b*) *e*-species are derived from *p*-species and it has been shown in the above discussion of *p*-species that production of *p*-species declined in Centrales during late Neogene (UM-UP), so that parents for *e*-species did not increase during that time in the same proportion as they did during the early subperiods of Tertiary. From (*a*) and (*b*) in the above sentence the difference between the calculated and observed numbers for total species might be explained: the facts of history for Centrales seem to have differed from a

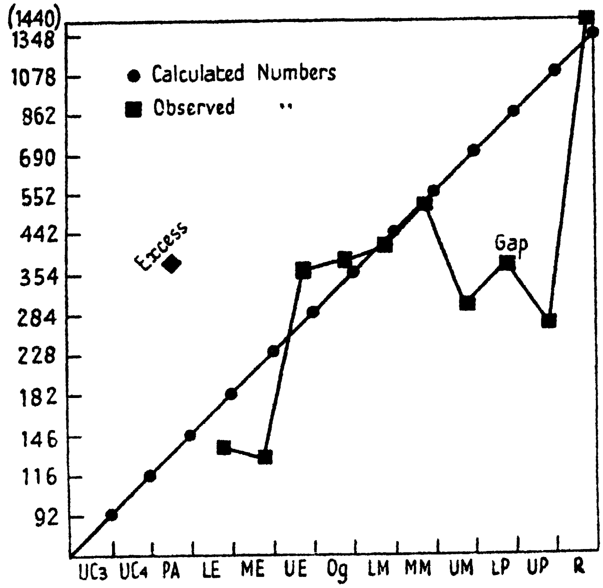

FIG. 4. Total numbers of species of Centrales occurring in different geological periods (for symbols see p. 95). The vertical scale is a geometric series described in the text.

plain geometric progression continuing beyond middle Miocene into Recent as was found for allogamous Pennales. These facts include extensive *incipient* decline in old genera, which shows as a decrease in the production of new *p*-species, particularly in late Neogene (UM-UP).

But the above explanations of the UM-UP gap seem to prove too much, because the same calculated series with its continued increase for total species-number arrives at 1,348 for Recent, which is a much closer approach to the 1,440 observed than any of the late Neogene values: and this 1,348 can be *calculated* as consisting of 674 old *p*-species, 168 new *p*-species, and 506 new *e*-species, from the formula for distribution—4 old *p*:1 new *p*:3 new *e* (see Table II below).

This raises another problem, one solution of which could be suggested to be that the late Neogene decline in production of *p*-species may apply only to certain very old genera and that the younger genera have compensated for this decline by producing enough *p*-species to maintain the general residual increase in all three types of species. The younger genera with known Tertiary records have not done this, but there are 38 centric genera which had only Recent records in the original analysis.

Another aspect, which definitely rules out this suggested solution, is that all the Recent-only (Ro) genera taken together include only about 89 species, so that they could not account for the difference of about 900 species between the 1,440 for Recent and the 523 for middle Miocene. Among the 38 Ro genera in Centrales there are 18 monotypes, 8 ditypes, 3 tritypes, 2 with 4 species, and one each with 5, 6, 7, 10, 11, 13, and 23 species. Allowing for recently found fossil records which transfer one of the tritypes, the 11-type, and the 23-type to the fossil-and-recent or FR class; this represents a total of 89 species in 35 Ro genera. The small size, of 1–4 species with a limit about 8, is characteristic of genera with a duration which has not been long, whether these genera are Recent only or short-lived extinct genera (Small, 1948*a*).

The numerical facts are that, to our present knowledge, the total number of Centrales reached 523 in Middle Miocene, varied through 299 UM, 359 LP, 260 UP, and rose to 1,440 in Recent; but in view of the regularities found for allogamous Pennales throughout Tertiary and for Centrales *p*-species from Paleocene to middle Miocene and for the total centric species from lower Eocene to middle Miocene, our present knowledge may not be the complete truth.

The only logical solution of this problem, which accounts for the 1,348–1,440 total species in Recent, is that we know only part of the late Neogene diatom flora for *old* genera, and that the remainder still lie hidden below the Recent diatom deposits at the bottom of the present oceans. This view is supported by the known upper Miocene and lower Pliocene quotas of new species, which quotas show approximations to a 3:1 *e*/*p* ratio thus: UM 30/6 and LP 79/28, which have deviations of only +3 and −1 from nearest approaches to 27/9 and 80/27 with totals of 36 and 107 respectively.

The argument here is that among the known species, as subperiod quotas for these two subperiods, the main ratio is approximately maintained; therefore we know neither all the *e*-species, nor the earlier than Recent records for many of the *p*-species which presumably originated in upper Miocene and lower Pliocene and now contribute to the known total of species which occur in Recent. The *e*-species would have begun and ended in these two subperiods, and do not now occur in the records of Recent species; but the *p*-species would have continued into Recent and given off their quota of new *p*-species and new *e*-species. All these must still be labelled Ro in the absence of fossil records for both parents and progeny.

TABLE II

Data for Centrales

	Calculated species-numbers*					Observed species-numbers				
Geological Period	Total *OC.*	Total *p*5:	Old *p*4:	New *p*1:	New *e*3	Total *OC.*	Total *p*	Old *p*	New *p*	New *e*
Recent	1,348	842	674	168	506	1,440	259	259	—	—
Upper Pliocene	1,078	674	539	135	404	260	259	257	2	1
Lower ,,	862	539	431	108	323	359	280	252	28	79
Upper Miocene	690	431	345	86	259	299	269	263	6	30
Middle ,,	552	345	276	69	207	523	309	255	54	214
Lower ,,	442	276	221	55	166	426	273	210	63	153
Oligocene	354	221	177	*44	133	372	228	184	*44	144
Upper Eocene	284	177	142	35	107	356	203	116	87	153
Middle ,,	228	142	114	28	86	127	119	105	14	8
Lower ,,	182	114	91	23	68	132	105	91	14	27
Paleocene	146	**91**	73	18	55	361	**91**	73?	18?	270
Upper Cretaceous 4	116	73	58	15	43	—	—	—	—	—
,, ,, 3	92	58	46	12	34	—	—	—	—	—

* Calculated from total *p*-species 91 in Paleocene, assuming regularity of the ratios given at the head of the columns. (See text.)

This should give an added interest to the examination of modern deep-sea cores which may penetrate the thick layers of modern deposits, and so reach down to the Pliocene and upper Miocene layers assumed to lie below these modern deposits. Deep-sea cores taken off the west coast of Africa, between Dakar and Walfish Bay, would probably have a still wider significance by penetrating through, or past, many Tertiary layers to pre-Tertiary deposits which have remained entirely submarine since the beginning of Cretaceous. These older beds seem to be exposed in some places, as if the strata lie tilted, below the sea. See Lohman (1941) for core technique.

Divergence from recognized rules or regularities always have special interest, particularly when they lead to further searches for explanations, either immediately or in the future. In the case of the divergences found in Fig. 4, immediate searches have disclosed the possible types of record which would tend to fill up the obvious gap, but the way is opened for future work which might remove the divergences altogether. The kinds of records to be expected from the examination of deep-sea cores are clearly indicated, namely, (*a*) a number of new fossil records for species now known only as Recent, and (*b*) about three times that number of records for species not now known at all.

The 'expected' numbers of species in these two categories are set out with the other data for Centrales which have been discussed above, in Table II. The divergences of the observed and calculated numbers for Centrales make this Table II less of a direct arithmetical demonstration of regularities than the Table I and Figs. 1 and 2 for allogamous Pennales; but the presence in the Centrales data of similar regularities and of divergences from these regularities are demonstrated by the above detailed analysis.

SUMMARY

1. The phenomena of quantitative evolution in diatoms are discussed in relation to the new outlook on evolution as shown in some recently published books and papers, particularly since 1941; and a large amount of agreement in general and in detail is demonstrated.

2. Just (1944) concluded his discussion thus: 'The rates of evolutionary processes differ so greatly that they can not be expressed in simple mathematical terms.' This is very true, and numerical analyses show a number of correlations which are interconnected in a complex manner.

3. Residual or overall increase in numbers of species is here shown to be correlated with the following:

(*a*) the phases of development of a group, and of its constituent genera;

(*b*) a rate of increase which has been more or less uniform during the *increase phase*, and which has been correlated with and controlled by—

(*c*) the rate of production of new species; and

(*d*) the proportion of new species which have been of the permanent type.

4. The factors 3 (*c*) and 3 (*d*) have been measured, and correlated with their resultant as 3 (*b*), in both the main groups of diatoms, thus:

For allogamous Pennales the speciation rate has been a proportion of about 16 new species from 9 old species (or 1·77) per subperiod of about 7 million years, and the proportion of permanents or *p*-species has been about 9 out of 16.

For Centrales the speciation rate has been a proportion of about 1 new species from 1 old species (or 1·0) per subperiod of about 7 million years, and the proportion of permanents or *p*-species has been about 4 out of 16.

5. There has been a marked difference in the arithmetical history of the two groups during Tertiary time, and in the end-products of these two histories.

6. Four phases of development of a group or genus are recognized, namely, an initial *lag phase*, an *increase phase*, a *stationary phase*, and a *decline phase*. The allogamous Pennales shows only the first two phases, and the Centrales has approached the *stationary* phase as a group, while showing all four phases in some of its constituent genera.

7. The end-products of the history of Centrales, with its many extinct genera and its elements of the *decline phase* in other old genera, in the form of the frequency distribution of generic sizes, are similar to those of some other groups of plants and animals.

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The Perithecia of *Ophiostoma Majus* (van Beyma) Goidànich

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With six Figures in the Text

INTRODUCTION

ACCOUNTS of perithecial formation in the genera *Ophiostoma* and *Ceratostomella* have been given by Saccardo (1882), von Hohnel (1918), Sydow (1919), Dade (1928), Nannfeldt (1932), Nannfeldt and Melin (1934), Davidson (1935), Andrus and Harter (1933, 1937), Andrus (1936), Goidànich (1935), and Gwynne-Vaughan and Broadhead (1936). These accounts suggest that there are fundamental differences between species in both genera and they differ in the interpretation of observations based on *C. fimbriata*. These differences are related mainly to the method of division of the ascogenous cells, the formation of asci, and the cleavage of ascospores, there being général agreement in the accounts of perithecial wall and neck formation. The present detailed examination of *O. majus* was undertaken since the method of perithecial formation differs in important details from that in other species under examination, or in published descriptions. Descriptions of the species under investigation will be published later. A detailed examination of all species of the two genera may indicate more satisfactory criteria for the determination of natural relationships than the characters of the perithecial wall and shape of the ascus on which the present classification is based.

MATERIALS AND METHODS

The strain of *O. majus* isolated from beech sawdust and used for a previous investigation (Hutchinson, 1939) was examined. Stock cultures were kept on prune agar slopes at room temperature, and at first were subcultured at monthly intervals. After 6 months fruiting became erratic and smaller perithecia were formed. Examples of these fruiting and non-fruiting cultures were then subcultured at intervals of 7 to 10 days. After 6 months of this frequent subculturing there was a noticeable increase in the number and size of perithecia formed. After 12 months production was abundant, and the perithecia formed were of similar size to those in the first isolations. Table I compares the size of the perithecia in the original isolations, those formed 6 months later, and the measurements given by van Beyma in the original description (1935), which was based on culture material. The Centraalbureau

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voor Schimmelcultures have compared cultures of this strain with the type culture of the species, and agree that it is *O. majus*.

TABLE I

	Strain under examination.		
	Measurements shortly after isolation.	Measurements after 6 months in culture.	Measurements by van Beyma of type culture.
Diameter .	600–1,000 μ	350–450 μ	320–480 μ
Neck . .	2·5–4·5 μ × 60–95 μ	2–3 μ × 60–70 μ	2–4 μ × 50–80 μ
Ostiolar hairs .	100–250 μ × 3–4 μ	200–250 μ × 4 μ	250–325 μ × 4 μ
Ascospores .		9·5–7·1 μ × 5·9–4·1 μ	7·3–8·3 μ × 3·4–4·7 μ

Material for examination was obtained from petri-dish cultures on prune agar kept at 27° C. These were examined at intervals, and portions of agar bearing the required stages cut out, fixed in Fleming's weak solution, and embedded in paraffin wax of m.p. 57° C. by use of cedar-wood oil. If softer waxes were used the brittle perithecial wall tended to fall apart during sectioning. Sections were cut from 2 to 7 μ in thickness and stained with Haidenhain's iron-alum-haematoxylin, which gave satisfactory preparations of older stages when used alone. Younger stages were counterstained with erythrosin in 90 per cent. alcohol, orange G in water, or light green in clove oil, erythrosin giving the clearer preparations. Gentian violet was used for the examination of the walls of the fertile cells.

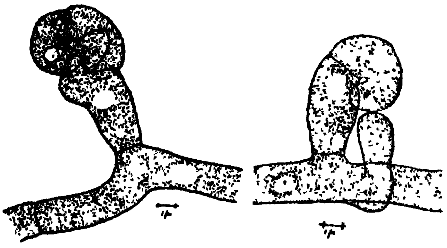

FIG. 1*a*

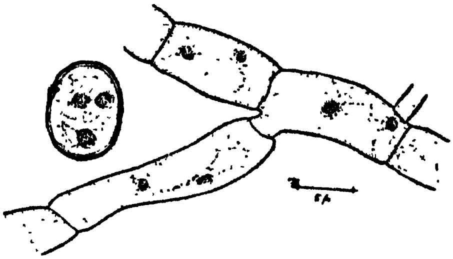

FIG. 1*b*

FIG. 1*a*. Unstained perithecial primordia formed on small vegetative hyphae in hanging-drop culture. FIG. 1*b*. A macroconidium and a portion of a mature vegetative mycelium showing the nuclear arrangement.

STRUCTURE AND DEVELOPMENT OF THE PERITHECIUM

Perithecia are formed after 4 to 5 days' growth at 27° C., normally in groups in localized areas. In petri-dish cultures they appear in the region of the inoculum after 4 to 5 days, occasionally around the edge of the plate after 10 days, and rarely appear elsewhere.

The perithecial primordium is similar to that described for other species, originating as a short, curved, lateral branch from a thin vegetative hypha. It becomes septate, and later is enveloped by outgrowths from its basal cells or from the vegetative hypha (Fig. 1*a*). Nuclear arrangement and possible cellular fusions of this stage have not been examined.

The enveloping branches form a wall which becomes differentiated into two zones. The outer zone is one to three cells thick, the cell-walls becoming slightly thickened and pigmented at an early stage. The inner zone is four to six cells thick, with thin walls and denser contents.

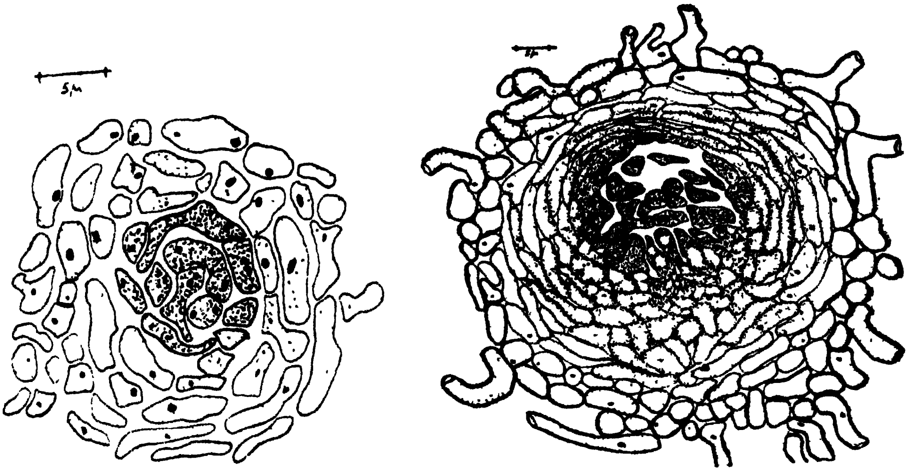

FIG. 2*a* FIG. 2*b*

FIG. 2*a*. Section of a young perithecium showing linear growth of the ascogonium to form a darkly staining central coil. FIG. 2*b*. Section of a young perithecium showing the start of cushion cell and of neck formation. The fertile cells show traces of a linear arrangement distorted by growth in the confined space in the centre of the perithecium.

The ascogonium divides to form a coil of multinucleate cells, with dense contents staining more readily than those of the wall cells (Fig. 2*a*). The nuclei are occasionally in pairs, but more usually are scattered irregularly. In some sections the coil appears to be in close contact with the wall, there being no indication of a central cavity. In other perithecia it is coiled freely within a central cavity, there being a distinct space between the wall and ascogenous cells which is not due to shrinkage during preparation. The formation of a similar initial coil has been reported for *C. moniliformis* (Andrus and Harter, 1937), *C. fimbriata* (Gwynne-Vaughan and Broadhead, 1936), and *C. multiannulata* (Andrus, 1936), but has not been found in other species. The coil later alters in form, due to a change from the original regular linear division to a more irregular type with a tendency towards centripetal multiplication. The connexion between the cells is easily broken. While some remain in irregular rows the arrangement usually becomes much distorted by continued growth and overcrowding in the confined space in the centre of the perithecium (Fig. 2*b*). In sections more than 5 μ thick these cells appear as an almost solid mass, those in the lower portion merging with the inner wall. The majority have dense contents, but a number begin to form large vacuoles. By this stage the walls of the cells in the outer zone of the perithecium have become thickened and brittle, and the cells in the inner zone have commenced to enlarge and become vacuolate. This enlargement begins in the cells at the base of the perithecium and extends upwards on each side. By the time the outer zone has developed into the thick carbonaceous wall characteristic of the genus, these enlarged cells of the inner zone have developed into a broad band of 'cushion cells' (Fig. 3).

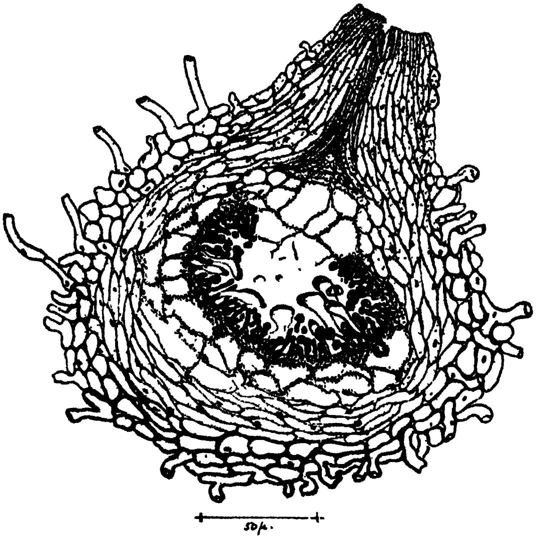

FIG. 3. Section of a perithecium showing the cup-shaped mass of fertile cells surrounded by a band of 'cushion cells'. The thin-walled tissue which originally filled the centre of the cup has begun to break down and to be displaced in the lower part of the cup by the club-shaped vacuolate ascogenous cells.

Further multiplication of the ascogenous cells takes place mainly at the sides of the mass, so that by the time the cushion cells have formed a complete investment they form a cup-shaped mass in the centre (Fig. 3). The tendency to radial arrangement is retained, though many irregularly scattered cells can also be seen. There is much variation in size, shape, and nuclear arrangement, but a gradual increase in the number of small binucleate cells becomes evident (Fig. 4*a*).

This division to form an irregular mass of multinucleate cells is similar to the corresponding stages in *C. Piceae* as described by Varitchak (1931), *C. fimbriata* as described by Mittman (1933), Andrus and Harter (1933), Gwynne-Vaughan and Broadhead (1936), and to *C. moniliformis* (Andrus and Harter, 1937) and *C. multiannulata* (Andrus, 1936) after the loss of the initial coil arrangement. These authors, however, disagree in the interpretation of their observations. Gwynne-Vaughan and Broadhead, and Varitchak, describe the divisions as resulting in 'ascogenous hyphae', though Varitchak states that these change form later in development and become difficult to identify. Elliott (1925) also interprets the cells of *C. fimbriata* as 'ascogenous hyphae', but it is noticeable that the drawings illustrating his article bear little resemblance to the microphotographs of similar stages of the same species figured by Gwynne-Vaughan and Broadhead. According to Andrus and Harter and to Mittman the loss of the cell-wall early in development strongly influences the further divisions of the ascogenous cells. They state that no definite hyphae are formed, though in *C. fimbriata* they report that the daughter-cells may cling together in 'rough chains'. This breakdown of the wall of the ascogenous cells is not found in *O. majus*. Though there is no distinct hyphal system after the initial coil has been lost, a definite wall can be demonstrated throughout the divisions leading to ascus formation. The walls of all cells within the outer layers of the perithecium are delicate but can be seen in a heavily stained preparation. The walls of the ascogenous cells exactly resemble those of the other thin-walled tissue in the perithecium, and of the vegetative hyphae, and are not lost until after maturation of the ascus. The irregular outline of the naked protoplast of the mature ascus is strikingly different from the outline of the young ascus, and from the outline of all cells before ascus formation (Fig. 6*g*). It is interesting to note that although Gwynne-Vaughan and Broadhead's description differs from that of Andrus and Harter, the microphotographs they publish are very similar to those of corresponding stages in *O. majus*. In both sets of illustrations distinct chains of cells appear, but no filaments resembling hyphae can be seen. In *O. majus* there is no indication of any localized growing-points, such as one would expect if hyphae were being formed, and the extremely loose connexion between the relatively unelongated daughter-cells is most unlike any normal hyphal system.

The irregular arrangement of the majority of nuclei is similar to the descriptions by more recent authors, there being no regular system of paired nuclei such as is drawn by Elliott (1925). The occasional 'pairs' are probably late stages in division, before the daughter-nuclei have moved apart.

Formation of the perithecial cavity. The formation of the cavity has received little attention from previous workers. It is detailed here for comparison with the method in another species which will be described in a later communication.

In the early stages the ascogenous cells occupy the whole of the centre of the perithecium, appearing as a solid mass in sections more than 5 μ in thickness. There is, however, a small amount of space above the cells, and between those in the lower portion (Fig. 2*b*). Later the centre becomes filled with a thin-walled tissue apparently continuous with the inner wall (Fig. 3). The vacuolate ascogenous cells already described enlarge greatly during development, their swollen heads displacing the thin-walled tissue at the sides and base of the hollow in the cup-shaped mass (Fig. 4*a*). Shortly before ascus formation the thin-walled tissue and the vacuolate cells begin to disintegrate (Fig. 4*b*). Breakdown is also apparent among the other ascogenous cells in the period immediately before ascus formation, some of the smaller ones not destined to form asci becoming vacuolate and losing their walls. By the time nuclear fusion has taken place in the ascus there is a large cavity in the centre of the perithecium, containing the disorganized remnants of these cells. During the maturation of the asci the cushion cells of the wall break down, the mature perithecium containing a mass of ascospores embedded in a mucilaginous mass.

This agrees with the findings by Andrus and Harter (1937) and Andrus (1936), who describe digestion of the inner wall and of certain ascogenous cells in *C. fimbriata*, *C. moniliformis*, *C. multiannulata*. Gwynne-Vaughan and Broadhead (1936) find no evidence of digestion in *C. fimbriata*, and attribute the enlargement of the cavity to loss of turgidity by the cushion cells. In *O. majus* the disorganization is due to digestion, there being a gradual breakdown of the cells rather than a sudden collapse due to loss of turgidity.

The large vacuolate multinucleate club-shaped cells which are formed during the period preceding ascus formation have not been described for other species of these genera. Andrus and Harter, however, describe fragile inflated and 'apparently uninucleate' cells preceding the asci in the centre of the perithecium, which are formed by certain types of division of the multinucleate fertile cells of *C. moniliformis*. In *O. majus* the club-shaped cells occupy the lower portion of the perithecial cavity shortly before ascus formation, the asci being formed between their lower ends. During the maturation of the asci these club-shaped cells disintegrate, their remnants joining the other disorganized tissue in the centre of the perithecial cavity. They are apparently analogous to paraphyses, though here formed by the ascogenous tissue. Brooks (1910) has described somewhat similar cells 'of irregular growth . . . poor in cytoplasm' developed from the ascogenous cells of *Gnomonia erythrostoma* in the period before ascus formation. He notes that they cannot be regarded as typical paraphyses, although they may take part in the formation of the cavity of the perithecium.

Formation of the neck. The first sign of neck formation is a slight increase in the density of the contents of some of the inner wall cells in the upper portion of the perithecium (Fig. *2b*). This becomes visible at about the same time as the first enlargement of the cushion cells. These inner wall cells elongate and divide rapidly, forming a cone of filaments projecting towards the outside of the perithecium. By continued growth they force themselves between the cells of the wall immediately above them and protrude to the exterior by about the time the fertile cells have formed a cup-shaped mass in the centre (Fig. 3). This is purely a mechanical action, as there is no indication of any breakdown of the displaced cells. By continued growth the typical long cylindrical neck is formed, and the outer cells become thickened and pigmented. The inner cells break down to form a cavity which is continuous with the cavity of the body of the perithecium. The cells lining the base of the neck cavity separate from each other to form a fringe of filaments around the inner orifice. The outer neck hyphae remain in close contact, overarching the top, until after maturation of the ascospores. They then separate at the tip, and bend outwards to form the ostiolar corona of filaments. This entirely agrees with all previous accounts of neck formation in the genus.

Formation and Development of the Asci

Asci are formed from the small binucleate cells, and cannot be distinguished from other ascogenous cells before nuclear fusion (Fig. 4*a*). There are no crozières, and in cases where the linear arrangement is not lost each of the cells in a chain may form an ascus (Fig. 5). This is similar to the method described for *C. fimbriata* by Elliott (1925) and Mittman (1933), and by Sartorius (1927) for *C. adiposum*, in that they agree that the ascus is formed without crozier formation. They differ in their accounts of the origin of these binucleate cells, Elliott and Sartorius finding them to be formed by the terminal septation of ascogenous hyphae, Mittman by the free division of isolated cells. In *O. majus* the mode of formation is intermediate between these two descriptions, some being irregularly arranged, but the majority growing in radial rows

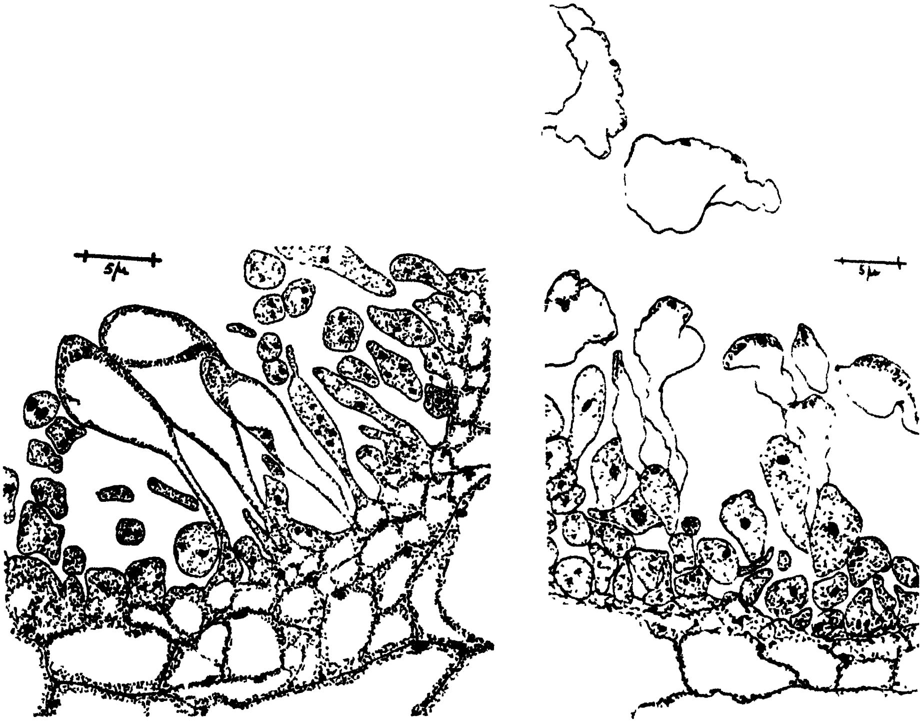

Fig. 4*a* Fig. 4*b*

Fig. 4*a*. Part of the fertile cells in a section of the perithecium shortly before nuclear fusion in the ascus. Binucleate asci are present among the large multinucleate vacuolate cells. Fig. 4*b*. Part of the fertile cells in a section of the perithecium after nuclear fusion in the ascus. Uninucleate asci are present, and the multinucleate vacuolate cells are beginning to break down.

towards the centre of the perithecium. The description of hook formation given by Andrus and Harter (1937) is related to the loss of the walls of the ascogenous cells, and is supported by camera-lucida drawings of isolated figures from their preparations. A detailed study of the mass of ascogenous cells in *O. majus* has failed to show such stages. Groups bearing some resemblance to these figures could be picked out very occasionally, but in such cases their limits were very difficult to determine and there was a possibility of the section having been cut through some of the cells of the group. Such groups were of rare occurrence, while many of the binucleate cells could be seen to be formed in irregular rows by direct division. The figure given by Gwynne-Vaughan and Broadhead (1936), a simple curved hypha with four terminal nuclei, is unconvincing evidence of crozier formation, and differs very much from Andrus and Harter's description of the same species. The cells of the vegetative mycelium and the macroconidia are multinucleate, the nuclei having a hyaline vesicle with a darkly staining lenticular nucleolus on one side (Fig. 1*b*). It has not been possible to distinguish the details of the nuclear structure in the cells of the young perithecium, owing to their small size and to the dense contents of the ascogenous cells. In these stages the nuclei are seen as undifferentiated black masses (Figs. 2 to 4*a*), but after fusion in the young ascus the single nucleus enlarges, and again shows a hyaline vesicle with a lateral nucleolus (Figs. 4*b*, 5). The uninucleate asci remain in this state for a long time, and are very common in sections of semi-mature perithecia. At the end of this resting stage the appearance of the nucleus alters. A number of darkly staining masses are formed in the vesicle, which tend to run in irregular strands from the nucleolus to the opposite side of the vesicle (Fig. 6*a*). In some asci these strands seem to run into a second mass of chromatic material at this side. The appearance of this mass, if present, is uneven, there being no sharp outline such as delimits the nucleolus, and it probably represents merely the appearance of the closely packed ends of the strands.

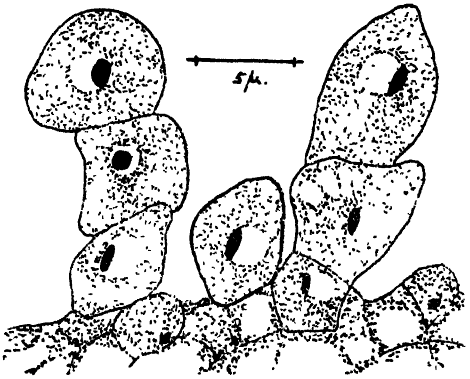

FIG. 5. Uninucleate asci showing linear arrangement.

Nuclear division takes place irregularly in the cytoplasm, and the daughter-nuclei are not grouped regularly in the centre of the ascus. The nuclei formed by the first and second divisions have a nucleolus and vesicle similar to the parent nucleus and lie quite freely in the cytoplasm (Fig. 6*b*, *c*). The nuclei during and for some time after the third division appear as minute black spots, only just visible under the $\frac{1}{12}$ in. oil immersion objective (Fig. 6*f*). The ascospores are formed by cleavage around the irregularly arranged nuclei after the third division. The nuclei increase to about two or three times their original diameter during the process and their vesicle and nucleolus can again be distinguished (Fig. 6*g*, *h*). The walls of the ascus break down during cleavage, the spores being held together in groups of eight by the residual protoplasm. They may break apart to be ejected singly from the ostiole, or remain in groups of two to eight. Spore formation takes place quickly once nuclear division has begun and there is little zonation of developmental stages in the perithecial cavity. This conventional course follows that described by

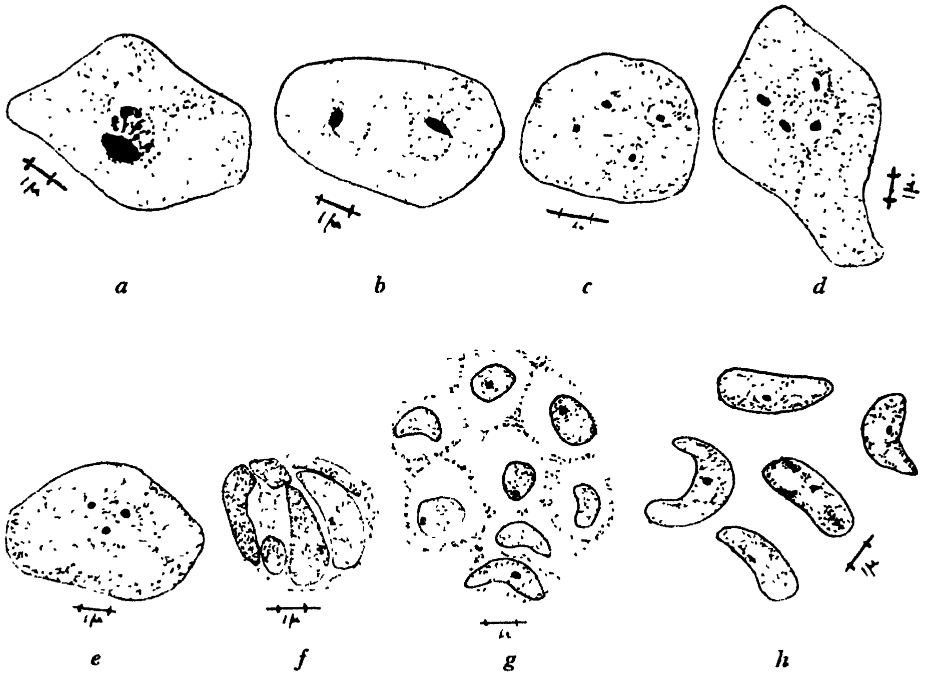

FIG. 6. Stages in nuclear division and spore formation in the ascus. *a*. Ascus in resting stage before the first nuclear division. *b*, *c*. Asci after the first and second nuclear divisions, showing the irregularly arranged daughter-nuclei, each with nucleolus and vesicle. *d*. Tetranucleate ascus in which the central grouping of the nuclei gives a slight resemblance to division within a central vacuole. *e*. Ascus showing nuclei in a central dense protoplasmic mass surrounded by a vacuolated layer. *f*. Ascus during spore cleavage, showing breakdown of the ascus wall, and very small spore nuclei. *g*. Section of an ascus at a later stage of spore cleavage, showing slightly enlarged nuclei and complete loss of the ascus wall. *h*. Young ascospores lying freely in the perithecial cavity.

Sartorius (1927) and by Gwynne-Vaughan and Broadhead (1936), there being no sign of division within the nucleoplasm (Elliott, 1923), or nuclear vesicle (Mittman, 1933; Andrus and Harter, 1937). The presence of a vesicle in all daughter-nuclei in the ascus supports the view that it is associated with each individual nucleus only, and that it takes no part in spore cleavage. The characteristic vacuoles which are formed during maturation of the ascus may occasionally aggregate in the outer portion to form a foamy layer surrounding a more dense central mass of protoplasm. If the nuclei chance to be in the central portion a superficial resemblance to some of Andrus and Harter's figures is given (Fig. 6*e*). The close association of nuclei with distinct vesicles may also give a superficial resemblance to divisions within a large vacuole (Fig. 6*d*).

These findings will be discussed in relation to other species in a later communication, but it is noted that the similarity between the general form of the perithecium and those of *C. fimbriata* and other species described by Andrus and Harter (1937) would lead one to expect similarity in the details of their development. There is, however, no sign of the peculiar method of spore formation within the vesicle of the original ascus nucleus. The presence of walls around all cells up to the time of spore formation shows resemblance to the account of *C. fimbriata* given by Gwynne-Vaughan and Broadhead (1936), although in *O. majus* the ascus wall breaks down during spore cleavage.

The tendency to linear septation seen at all stages up to the initiation of the asci indicates a relationship to normal ascogenous hypha formation. The loose connexion between the relatively unelongated daughter-cells and the absence of definite growing-points suggests, however, that 'ascogenous cells' is a more suitable description of the mass.

SUMMARY

1. A description is given of the details of perithecial formation in a new isolation of *Ophiostoma majus*, and the findings are compared with previous accounts of species of *Ophiostoma* and *Ceratostomella.*

2. It is shown that the ascogonium divides to form a compact mass of multinucleate ascogenous cells. These do not form definite growing-points, but a tendency towards linear septation can be seen.

3. The presence of a distinct wall is demonstrated in all cells within the perithecium up to the time of maturation of the asci. The mature ascus is shown to be deliquescent, the wall disintegrating during cleavage.

4. The nuclear vesicle is shown to play no part in spore wall formation.

5. The formation of the perithecial cavity is shown to be by the digestion of certain cells within the perithecium during the formation of the asci, combined with the breakdown of the cushion cells of the wall at a later stage.

6. A variation in perithecial size is described which is greater than that seen by van Beyma in the original description of the species.

ACKNOWLEDGEMENT

The author wishes to thank Professor MacGregor Skene and the staff of the Botany Department of the University of Bristol for much assistance in the earlier part of this work, and Professor John Walton of the Botany Department of the University of Glasgow for facilities for its completion.

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Studies in the Genus *Eremascus*

I. The Rediscovery of *Eremascus albus* Eidam and Some New Observations concerning its Life-history and Cytology

BY

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With Plates III and IV, and ten Figures in the Text

I. INTRODUCTION

IN 1883 Eidam reported the discovery of a new genus of ascomycete moulds in which naked asci, formed by fusion of two equal cells borne on short entwined hyphae, were produced singly throughout the mycelium. The fungus, which grew with other contaminants in a discarded flask of malt extract, was named *Eremascus albus*, the type species of a new genus. No cytological or physiological investigations were reported in Eidam's paper, and the fungus was lost before further work could be undertaken. Later, Stoppel (1907) described a second species, *E. fertilis*, found on the surface of apple-jelly, which closely resembled Eidam's fungus save for differences in spore shape and reproductive details. She investigated its cytology, as did also Guilliermond (1909), whose work extended our knowledge of parthenogenesis in this species. No other literature dealing with experimental work on *Eremascus* had appeared, nor had Eidam's species been found again, when the present investigation began. The studies reported here are concerned with new information about the life-history and cytology of *E. albus*; a second paper dealing with the environmental factors which control the occurrence of this rare species is in course of preparation.

In December 1944 Professor F. T. Brooks, of the Cambridge Botany School, received from Sir Frank Engledow a tin of mustard powder which was caked together and permeated throughout by a practically pure culture of a white mould; the mustard had been in home storage for a long time, perhaps for years. Examination showed the presence of numerous 8-spored asci freely borne without any kind of investment. Preliminary cultures (isolate 1) demonstrated that the asci were formed by the fusion of two equal cells at the end of short entwined hyphae and that the asci occurred over the whole mycelium. He was reminded of Eidam's figures of *E. albus* (1883) and provisionally identified the mould as this species. He then asked friends for other old samples of household mustard. Two, provided by Sir Rowland Biffen, also contained a similar white mould, but one was so heavily contaminated by Penicillia and Aspergilli that a pure culture could not be obtained. The other

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sample gave isolate 2, which agreed entirely with isolate 1. In addition, another white mould (isolate 3), producing chains of arthrospores, was isolated from these mustard samples, but at the time it was uncertain whether it was a distinct fungus or a phase in the life-history of what was presumed to be *E. albus*. Isolate 3 never produced asci and was subsequently shown to have no connexion with *E. albus*; it awaits identification and will not be considered in detail in this paper.

Isolates 1 and 2 grew very slowly on ordinary media such as Dox's and 2 per cent. malt agar. Mr. J. Bean (Professor Brooks's technical assistant) found that they grew appreciably better on malt agar to which 40 per cent. sucrose had been added; the cultures were then maintained on this medium, which will be referred to as malt-sucrose agar.

In July 1945 Professor Brooks kindly handed all the cultures to me for further investigation, it being suggested that isolates 1 and 2 were actually the long-lost *E. albus*. It was soon found that the addition of 0·5 per cent. Difco yeast extract to the malt-sucrose medium enhanced growth and sporulation; this medium will be referred to as malt-sucrose-yeast agar. The present study aims at providing a more complete account of the development and structure of *E. albus*, and of the environmental factors which control its growth. It is influenced by the osmotic pressure of the medium and by relative humidity in an unusual fashion, being intolerant of saturated conditions; data concerning this aspect of the investigation will shortly be presented in a second paper.

II. FURTHER ISOLATIONS OF *EREMASCUS ALBUS*

Three new isolates of *E. albus* were obtained during the routine examination of substrates for the possible presence of this apparently rare species. In December 1947 several colonies of *E. albus* were discovered on the surface of mincemeat (isolate 4), and in April 1948 a further isolate was obtained from stored greengage jam where the mould was present as a pale yellowish mat on the surface (isolate 5). The white colonies on the mincemeat gradually turned pale yellow as they aged, and this fact, coupled with the yellow colour on the jam, provides an interesting comparison with the development of a characteristic yellow colour on malt-sucrose-yeast agar which is described later.

In a series of papers on mould deterioration of feeding-stuffs in relation to the humidity of storage, Snow et al. (1944) and Snow (1945) noted that a few moulds, especially those of the *Aspergillus glaucus* group, would develop at relative humidities of 75 per cent. and below. Although growth was very slow, a few moulds developed on locust beans stored for two years at 65 per cent. R.H. Samples of household mustard were set up at low humidities (76 and 80 per cent. R.H.) and examined at intervals for the possible appearance of *E. albus* among any moulds developing on them. Experiments had shown that isolates 1 and 3 could grow at these humidities, and since Eidam's record in 1883 was the only one for the species before the present isolations were made, the demonstration of its occurrence in household mustard as a possible contaminant was thought to be of interest. The jars containing the mustard samples (taken from locally purchased tins of mustard powder) were incubated at 25° C. over salt solutions giving the required humidities and examined at monthly intervals. The dominant mould was a white-spored *Aspergillus*, but in addition to this, chains of conidia resembling those associated with isolate 3 and asci of *E. albus* were also seen after about 3 months in one of the six samples examined. Development was allowed to continue for 12 months when isolations from portions of the mustard bearing the greatest development of asci were made. Finally, pure cultures were obtained of the two fungi corresponding to isolate 1 (i.e. *E. albus*) and isolate 3 respectively. This culture of *E. albus* is isolate 6.

Pure lines of each isolate were set up from single ascospores isolated by means of a micropipette mounted on a Zeiss micromanipulator. All strains proved to be homothallic. The details of the isolations of *E. albus* may be summarized as follows:

Substrate.	Isolation and locality.	Number.
Drying malt extract	E. Eidam, 1883, Germany	Type species; lost
Household mustard powder	F. T. Brooks, 1944, Cambridge	Isolate 1
	,, ,, ,, ,,	Isolate 2
	C. E. Harrold, 1948, Cambridge	Isolate 6
Mincemeat	,, ,, ,, London	Isolate 4
Greengage jam	,, ,, ,, Cambridge	Isolate 5

III. THE LIFE-HISTORY OF *EREMASCUS ALBUS*

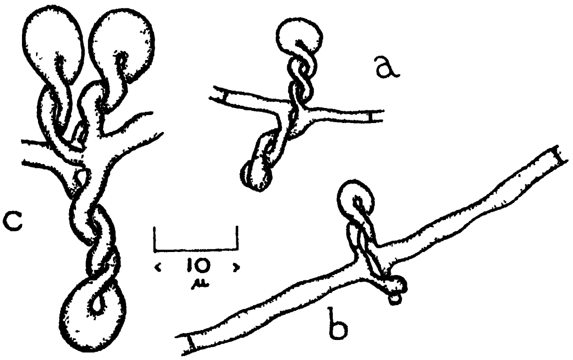
a. Slide culture technique

It was necessary to observe the details of ascus formation in the present isolates in order to confirm their identification as *E. albus*, and a slide culture technique was used for this purpose.

The method of Talbot (1945) was modified since an abundance of spores was not available. With a flamed wire loop, a drop of sterile water was placed on a clean flamed slide, and a sterile needle, previously touched on the surface of a sporing culture of *E. albus*, was stirred into this drop to produce a spore suspension. A drop of malt-sucrose agar was placed on a flamed coverslip and this was carefully lowered on to the inoculum drop. The two drops combined, holding the coverslip firmly to the slide with the seeded culture medium spread between the two. The size of the water and agar drops was adjusted so that approximately half the area beneath the coverslip was occupied by the culture medium. Such slide cultures were then incubated in sterile Petri dishes at 25° C. on glass triangles over a 16 per cent. saline solution (approximately 90 per cent. R.H.). This procedure was necessary since the use of water produced saturated conditions which prevent the development of *E. albus*; this effect will be considered in a later paper. The fungus develops and fruits rapidly in such slide cultures. Hyphae and asci stand out clearly in a colourless matrix of agar, and septa in the hyphae are clearly visible. Observations were carried out at intervals to ascertain the time required for the production of ascospores after the inception of ascus-formation, and the structures involved could be photographed *in situ* at different stages of development. Contamination of such cultures was very rare even though subjected to periodical examination under laboratory conditions.

b. Germination

Ascospores on malt-sucrose agar in a hanging-drop, over 16 per cent. saline solution at 25° C., germinated within 18 hours. Eidam (1883) and Stoppel (1907) stated that germination in their species required 2 days, but did not record their experimental conditions. Since temperature and humidity variations markedly influence germination time (thus germination of ascospores of the present isolates took 4 days at 25° C. and 80 per cent. R.H.), we cannot regard the discrepancy as important.



TEXT-FIG. 1. *E. albus*: coiling and fusion of gametangia during ascus formation.

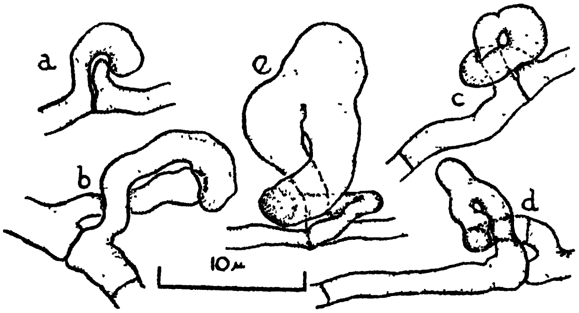
Germination is brought about by the splitting of the exosporium at one end of the almost spherical ascospore. The endosporium protrudes at this point to form a swollen vesicle from which one or more germ-tubes develop (Pl. III, *a*). Eidam (1883) records the production of germ-tubes at each end of the ascospore, but this has only rarely been seen in my preparations.

c. The normal process of ascus formation in Eremascus albus

Ascospores are delimited inside a spherical ascus produced by the fusion of two coiling gametangia (Text-fig. 1; Pl. III, *b*, *c*, *d*). The number of coils varies but is commonly more than one, whereas in *E. fertilis* coiling rarely occurs between the gametangia. Groups of gametangia are common and they arise from different points around the same part of a hypha, as in Eidam's original figures for *E. albus* (his Fig. 1, *c*). The two gametangia of a pair always arise from adjacent cells close to the dividing septum.

Eidam (1883) stated that the apices of the coiled gametangia were delimited from the basal regions before their fusion; in *E. fertilis*, however, fusion takes place without such prior delimitation of fusion cells (Stoppel, 1907). The position in the present isolates is intermediate since both conditions have been recorded. Thus, out of 23 asci in which development was closely watched 14 instances of fusion without previous septation of the gametangia were noted (Text-fig. 1, *a*, *c*), while only in two gametangial coils was there indubitable septation prior to fusion (Text-fig. 1, *b*). Examination of the present isolates revealed structures comparable with all those figured by Eidam (1883) except for his figure of 8-spored asci produced singly at the ends of hyphae. The variation in coiling and septation of the gametangia is shown in Text-fig. 1. Pl. III, *d* illustrates three stages in ascus formation; the gametangial fusion, the swelling of the ascus at the point of fusion, and finally, the delimitation of the ascospores.

The original description of *E. albus* contains no estimate of the time required for ascospore-formation. Periodical observation of selected asci in slide cultures showed that the process of ascus formation, from the production of gametangia to the delimitation of the ascospores, takes 5–6 days at 22·5° C. The ascospores are not dispersed by any special mechanism; the ascus wall disintegrates leaving eight ascospores adhering together in a cluster; such clusters may persist for long periods.



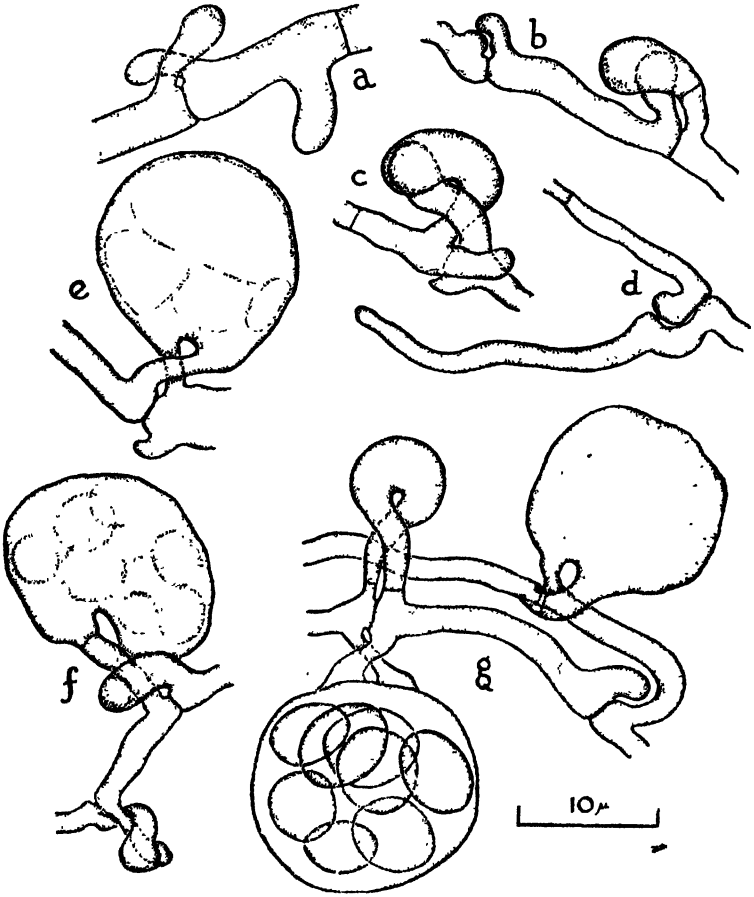
TEXT-FIG. 2. *E. albus*: anisogamous fusions.

d. Variations in ascus formation

The normal process of ascus formation in *E. albus* involves an isogamous fusion between two gametangia, but many variations have been observed which are of interest in discussing the relationships between *E. albus* and other members of the Endomycetales and Hemiascomycetes.

1. *Anisogamous fusion.* Instead of producing two equal gametangia the pairs of cells responsible for gametangium formation often put out unequal gametangia which, nevertheless, fuse to produce normal asci. Text-fig. 2, *a* illustrates the extreme case in which a larger gametangium completely dominates its smaller partner, while Text-fig. 2, *b*, *c*, *d* show similar anisogamy in which a certain degree of gametangial coiling is also present. Text-fig. 2, *e* illustrates ascus formation involving a smaller centrally placed gametangium about which is coiled a larger one, a situation highly suggestive of the condition found in Gymnoascus and Ctenomyces among the Gymnoascaceae. Usually the position of ascus formation in both isogamous and anisogamous

fusions is at the region of fusion and not displaced to one side as in Text-fig. 2, *c*. Such displacement of ascus formation coupled with anisogamy, as found exceptionally in *E. albus*, suggests the manner in which the more specialized antheridia and ascogonia in the higher Ascomycetes may have evolved from an isogamous ancestry.

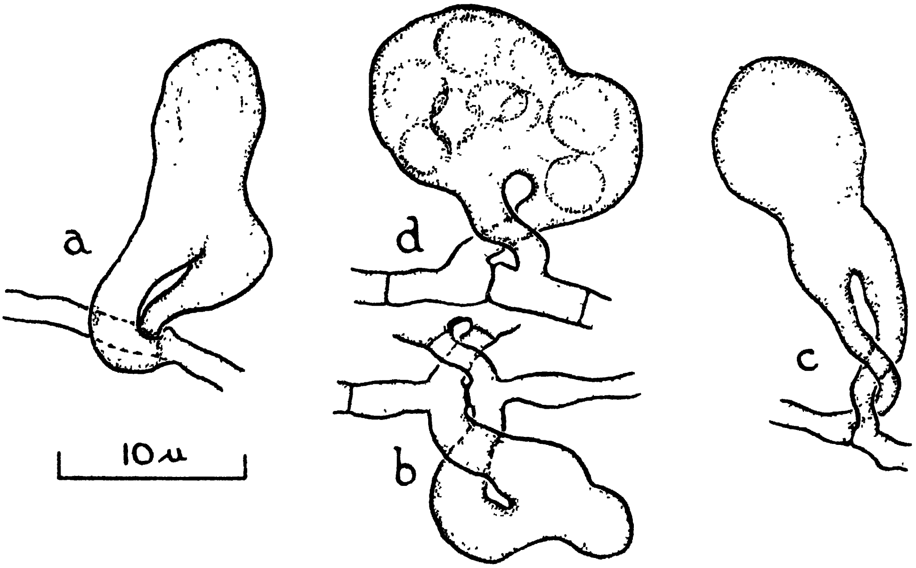


TEXT-FIG. 3. *E. albus*: anomalous development of gametangia.

2. *Anomalous development of gametangia.* While it is common for many pairs of gametangia to arise from the same two parent cells (Text-fig. 1, *c*), they usually do so close to each other at the septum separating the two cells. Occasionally, however, a cell which has produced a gametangium at one end will develop another at its opposite end without any septation of the cell taking place. Text-fig. 3, *b* shows the beginning of such a condition, while in Text-fig. 3, *f* we see a more advanced stage in which a cell having already produced a gametangium involved in ascus formation has given rise to another at its opposite end, which in turn is undergoing copulation. It may be remarked

that in the situation recorded in Text-fig. 3, *f* the smaller partners of both fusions arise from the same cell.

Sometimes a pair of cells having already undergone gametangial formation develops new outgrowths which appear to have no sexual function (Text-fig. 3, *e*). Text-fig. 3, *a* shows a pair of cells which have produced structures resembling gametangia but in which coiling ceased early and the gametangia grew on as sterile hyphae; a similar situation is recorded in Text-fig. 3, *c*, where the smaller gametangia lost their functions and underwent no subsequent coiling or fusion.



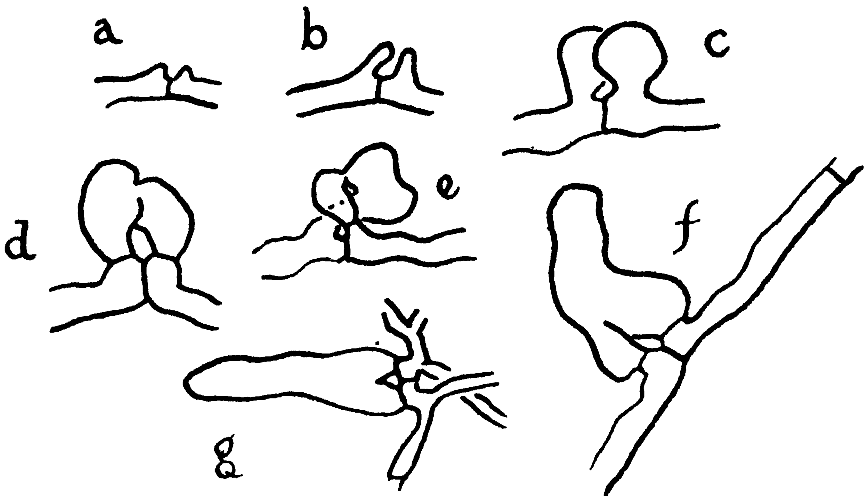
TEXT-FIG. 4. *E. albus*: variations in ascus shape.

Rarely, one member of a gametangial pair ceases to function as a gametangium and continues its growth as a hypha (Text-fig. 3, *d*). Text-fig. 3, *g* shows an example of a gametangium which had developed into a hypha and later produced gametangia and an ascus.

3. *Variations in ascus shape and ascospore number.* Eidam (1883) made no mention of any departure from the normal spherical ascus shape nor of any variation in the normal number of eight ascospores per ascus. Examination of both Petri dish and slide cultures disclosed the occurrence of certain variations bearing an interesting resemblance to the sexual process in *Dipodascus albidus* (Lagerheim, 1892; Juel, 1902). In *D. albidus* there is a fusion of two multinucleate gametangia leading to the production of an elongate multispored ascus (Text-fig. 5). Fusions in *E. albus* reminiscent of certain illustrations in Lagerheim's paper are shown in Text-fig. 4 (cf. the formation of an elongate ascus in *E. albus*, Text-fig. 4, *a*, *b*, *c* with Text-fig. 5, *f*). Cytological preparations of these unusual structures in *E. albus* were not obtained, but they are

presumably uninucleate, whereas those of *Dipodascus albidus* are multinucleate.

Sometimes there is a reduction in the number of ascospores per ascus, as shown in Text-fig. 6, *c*. This is probably due to abortion of some of the eight spores originally formed. Text-fig. 6, *a* shows six fully formed ascospores with two others incompletely developed. Continued division of the eight nuclei resulting from the normal division of the fusion nucleus in the ascus leads to the formation of many ascospores above the normal complement of eight. Such ascospores, as shown in Text-fig. 6, *b*, are smaller than usual but they germinate and otherwise behave normally. Similar reduction and increase in the number of ascospores per ascus were also observed in cultures of *E. fertilis*. Ascospore formation in the multispored asci of the species of Eremascus is by free-cell formation typical of the Ascomycetes and not by continuous cleavage characteristic of the sporangia of Phycomycetes.



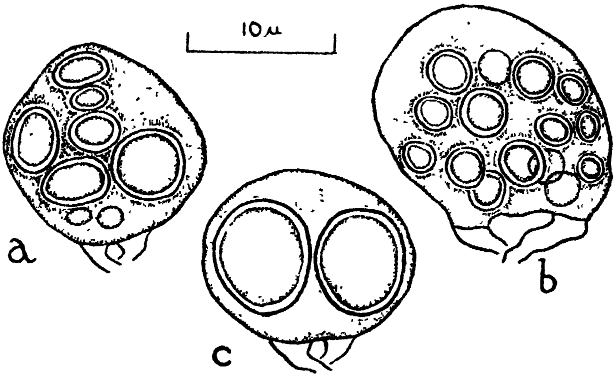
TEXT-FIG. 5. *Dipodascus albidus*: stages in ascus-formation. (After Lagerheim, 1892.)

e. The Thallospores of Eremascus albus

The original accounts of *E. albus* and *E. fertilis* describe no spore form other than the ascospore; in the present isolates, however, another structure referred to below as a thallospore (Mason 1933, pp. 6–7) was constantly found.

During thallospore development the tip of a hypha becomes swollen and assumes a spherical shape (Text-fig. 7, *a–c*), after which it is cut off by a septum from the hypha (Text-fig. 7, *d*, *e*). The significance of a double septum which not uncommonly occurs is unknown. Later the wall of the thallospore thickens (Text-fig. 7, *f–l*), and not infrequently the thallospore shape is elongate (Text-fig. 7, *j*, *k*). Intercalary thallospores are also found (Text-fig. 7, *l*). Production of thallospores is sometimes associated with the gametangia, one member of a coiling pair growing out considerably and cutting off a thallospore at its apex. Most remarkable, however, is the production of such spores at the apices of germ-tubes in many instances. Small isolated portions of agar are often present in the slide cultures referred to above, and in these ascospores often germinate and form thallospores directly with no development of mycelium; similar development has also been observed in hanging-drop cultures (Pl. III, *f*). These spores are cut off within 7 days of spore germination.

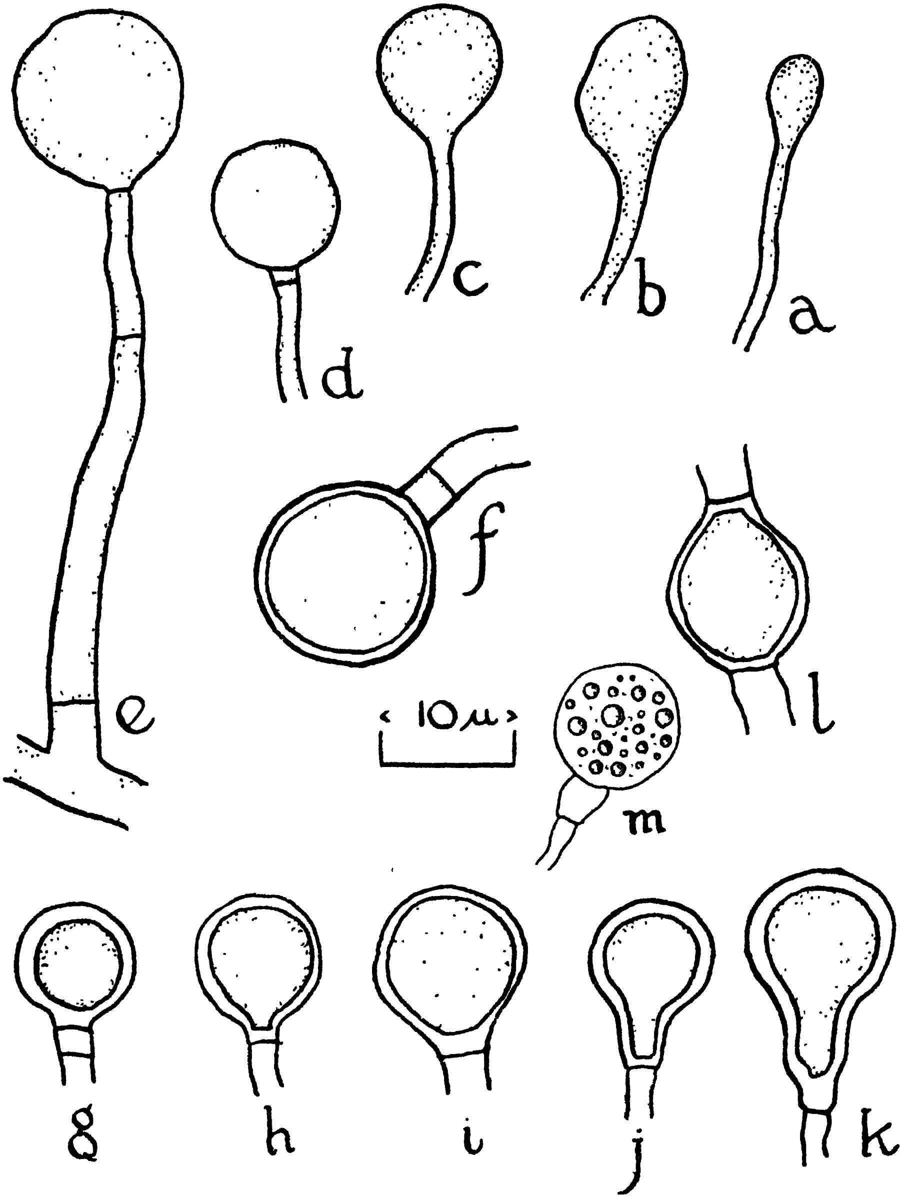
The conditions influencing the formation of thallospores are not clear. Temperature and pH variations which adversely affect growth had no effect on their occurrence. Colonies grown at low humidities, e.g. 76 per cent. R.H., developed very slowly, ascus formation being delayed for some weeks, but while thallospores were always present in such cultures, they were in no special abundance.



TEXT-FIG. 6. *E. albus*: variation in spore number per ascus.

The method of dissemination of the thallospores and their exact function in the life-history of the fungus are also uncertain. In old slide cultures the hyphae just below the thallospores dissolve away leaving them free. Attempts to germinate thallospores isolated by micromanipulation were unsuccessful. The thick wall of the thallospore suggests a resting function, perhaps tiding the species through severe conditions; however, the ascospores themselves are still viable after 3 years, the age of the oldest spores available for test.

Eidam (1883, his Fig. 24) recorded a parthenogenetic ascus borne at the end of a hypha, and it was thought possible that the thallospores in the present isolates might be such structures. No delimitation of ascospores was ever observed, however, and they are not considered to be concerned with ascospore formation in any way. Eidam's Fig. 25 (reproduced here as Text-fig. 7, *m*) bears a close resemblance to the thallospores in the present material (Pl. III, *e*). This structure, regarded by Eidam as an abortive parthenogenetic ascus, might conceivably have been a thallospore comparable to those found



Text-fig. 7. *E. albus*: thallospore formation. (*m* is after Eidam, 1883, and is not on the same scale as the other figures. It is approximately 18 μ in diameter.)

in the new isolates. The absence of a thickened wall can be accounted for by the impermanence of his material. The thallospores in the present isolates of *E. albus* have an average diameter of about 10 μ with a range of 7–17 μ. The structure represented in Eidam's Fig. 25 has a diameter of about 18 μ, so that the size is comparable.

f. Ascospore formation in the hyphae of Eremascus albus

Eidam (1883) recorded rare instances of abnormal ascospore formation, and figured ascospores in an ascus formed without the production of gametangia and situated at the end of a row of enlarged hyphal cells (his Fig. 24). Such asci were spherical and of normal size. Similar terminal asci were described by Stoppel (1907), but she decided that such structures in *E. fertilis* were not parthenogenetic in origin but arose from a normal gametangial copulation in which observation of the gametangia was difficult. She did, however, record parthenogenetic ascus development and development of ascospores in swollen hyphal cells, which was also observed by Guilliermond (1909).

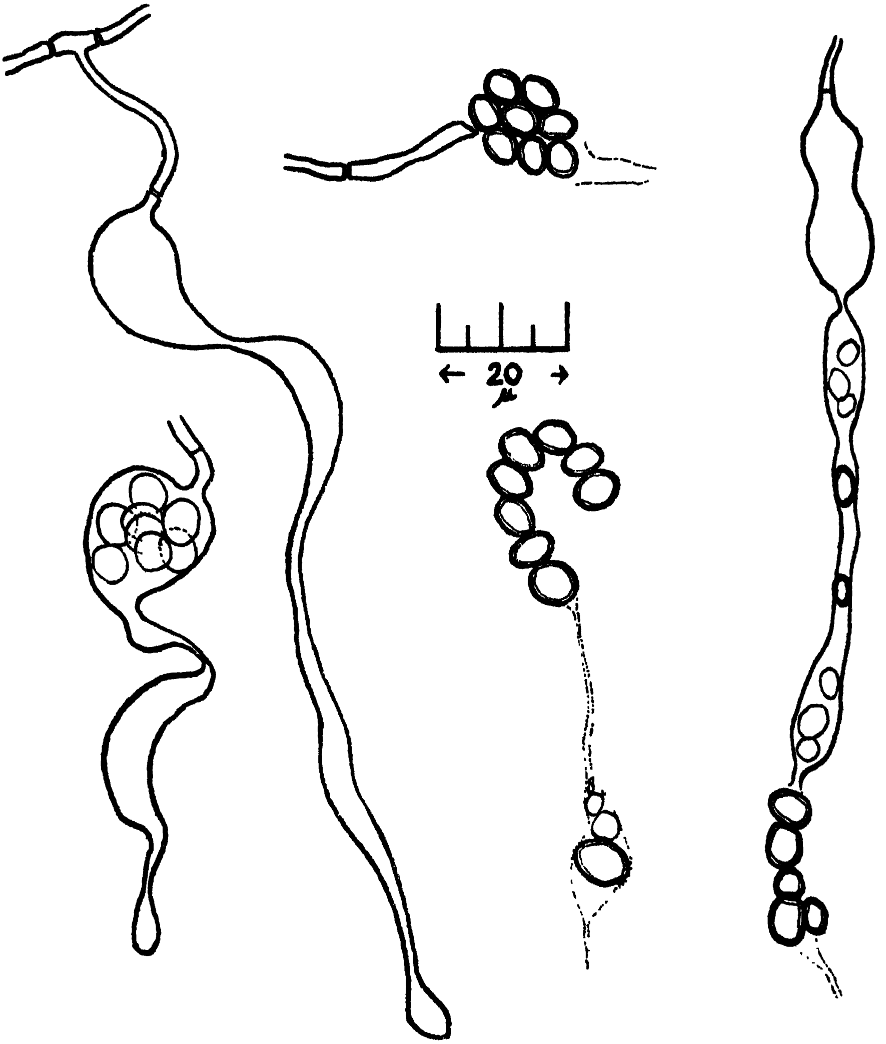
In older cultures of the present isolates of *E. albus* ascospores were formed in enlarged cells of some hyphae. Eidam's account makes no mention of such a process, although Fig. 4 in his paper shows hyphae with numerous intercalary and terminal swellings which may correspond to those associated with abnormal ascospore formation in the present material. There is wide variation in the number and grouping of the ascospores during such anomalous development (Text-fig. 8; Pl. III, *g*); the ascospores may be delimited in a single row or grouped together as in normal asci. In many instances irregular swelling of the hyphae without septa-formation takes place. When less than eight spores are present, small abortive spores can often be seen alongside the others which may or may not be greatly enlarged.

It has not been possible to obtain adequately stained preparations of hyphae undergoing abnormal ascospore development; and since no details of nuclear behaviour during this process are known, it is impossible to decide whether such development is due to true parthenogenesis or the fusion of two nuclei inside a hyphal cell. The ascospores in the hyphae are often in groups of eight, and this is regarded as presumptive evidence for supposing that a nuclear fusion has taken place in the hyphal cells followed by meiosis and then mitosis as in normal ascus development.

This situation in *E. albus* is closely paralleled in *E. fertilis*; Guilliermond's Fig. 42 (1909) shows a hypha with a series of aseptate swellings containing ascospores in varying numbers, closely resembling those in the new isolates of *E. albus*. In *E. fertilis* the gametangia commonly do not fuse, or may arise singly, and swell up into parthenogenetic asci containing 4 to 8 ascospores. Development of asci from single gametangia or from gametangia which have not fused was not observed in the present cultures of *E. albus* nor was such development described by Eidam (1883). No terminal asci corresponding to those found by Eidam were found in the new isolates. Beyond the fact that it is only in old cultures (upwards of two months old) that ascospores are produced in the hyphae, nothing is known of the exact conditions influencing such development.

Biggs (1937) describes the development of abnormally small intercalary asci in cultures of *Dipodascus uninucleatus* grown on a 1 per cent. lactose agar.

She recorded 'undifferentiated hyphal cells which may contain less than eight spores', and noted that 'in some cases there is evidence of a previous fusion of cells in the formation of these asci, but the smaller ones may be developed parthenogenetically'; her figures bear a striking resemblance to the comparable situation in *E. albus*. *Dipodascus*, *Eremascus*, and *Endomyces*, therefore, all exhibit ascospore formation unassociated with gametangial fusion, so that the phenomenon is apparently widespread in the Endomycetales.



TEXT-FIG. 8. *E. albus*: ascospore formation in hyphae. (Dotted lines represent the hyphae disintegrating to liberate the ascospores.)

IV. Taxonomic Characters of the Species of *Eremascus*

Cultures of the new isolates ascribed to *E. albus* were compared with cultures of *E. fertilis* during the course of identification. Three cultures of *E. fertilis* from the following sources were obtained for this purpose:

A: from honeycomb; isolated by Maurizio, 1939.
B: from shortcake; isolated by Stoppel, 1941.
(A and B came from the Centraalbureau voor Schimmelcultures, Baarn.)
C: from plum jam; isolated by the writer in Cambridge, 1948.

Several new facts concerning the cultural characters and ascospore size in both species came to light during the comparison which confirmed the original identification of the new isolates on the basis of gametangial characters. Thus, coiling of the gametangia in *E. albus* was frequent, but rarely occurred in *E. fertilis*. The almost spherical ascospores of the new isolates, apart from C above, also resembled the ascospore shape of Eidam's species rather than the smaller elliptical spores of *E. fertilis*.

The specific differences between *E. albus* and *E. fertilis* according to the original accounts (Eidam, 1883; Stoppel, 1907) are:

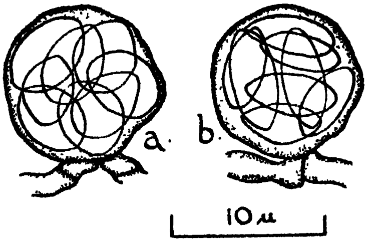
	Ascospore size.	No. of gametangial coils.
E. albus .	5·2 5·5 μ	1–4
E. fertilis .	5·2 · 3 μ	1, rarely more, often none

E. albus also produces groups of asci on the same part of a hypha, although each ascus is developed on an independent pair of gametangia. Stoppel (1907) and Guilliermond (1909) do not show more than two asci arising on the same part of a hypha, and this is confirmed in the present isolates of this species.

The malt-sucrose agars used to maintain the present isolates of *E. albus* and *E. fertilis* represent a considerable advance on the media used by Eidam and Stoppel. *E. albus* will not grow on malt agar alone unless the humidity is reduced to about 98 per cent. R.H. or below, and *E. fertilis* produces only a slow-growing corrugated colony on this medium (Pl. IV, *i*, *a*). Both moulds produce a normal convex colony when grown on a malt-sucrose agar; they have well-developed hyphae, and they have retained their characters through numerous sub-cultures during 3 years. The following characters appear to be constant in the two species. (The standards used in describing colony colour are those found in the Horticultural Colour Chart issued by the British Colour Council in collaboration with the Royal Horticultural Society. Equivalent colours on the Ridgway Scale are given where possible.)

Eremascus albus—colony characters. On malt–40 per cent. sucrose agar a pure white colony is formed; at first convex but later becoming concave as the aerial mycelium dies down. Finally, the entire colony surface is covered with a mass of liberated ascospores and assumes a powdery texture (Pl. III, *h*). The same characters are found in colonies on malt–40 per cent. sucrose–0·5 per cent. Difco yeast agar, but the growth rate and sporulation are increased; as the aerial mycelium dies down the colonies become yellowish. This colour was present in colonies growing naturally on mincemeat and jam substrates (isolates 4 and 5) and is a Primrose Yellow (60. 1/3 = Pale Lemon Yellow, 23. B Ridgway).

Ascospore size. The ascospores of *E. albus* are almost spherical while those in *E. fertilis* are elliptical with pointed ends. Strain B of *E. fertilis* showed wide variation in ascospore shape, some of the spores resembling those of *E. albus* rather than those of typical *E. fertilis* (Text-fig. 9). Ascospore measurements of both species were made from the original material where available and from 3-week cultures on different media at 25° C. From each culture samples of 200 spores (mounted in water) were measured; details of the average measurements are given below in Tables I and II.



TEXT-FIG. 9. *E. fertilis*: culture B, showing wide variation in ascospore shape in asci on the same mycelium.

TABLE I

Average Ascospore Size in Eremascus albus

Isolate.	Substrate.	Ascospore size (µ).
1	Mustard powder	7·5 × 6·3
	Dried M (at 90% R.H.)	7·5 × 6·3
1	M 40	7·5 × 6·3
	M 40 Y	7·5 × 6·3
2	M 40	8·1 × 6·9
	M 40 Y	7·5 × 6·9
4	Mincemeat	7·5 × 6·9
	M 40 Y	8·1 × 6·3
5	Jam	8·1 × 6·9
	M 40 Y	7·5 × 6·9
6	M 40 Y	7·5 × 6·3

M = 2% malt extract agar; M 40 = 2% malt-40% sucrose agar; M 40 Y = 2% malt–40% sucrose–0·5% Difco yeast agar.

The average spore dimensions of *E. albus* are 7·7 × 6·5 µ, with the size 7·5 × 6·3 µ as the most frequently occurring class. The range lies between 3·8–11·5 µ in length and 3·1–10·0 µ in breadth, these extremes being accounted for by the occurrence of single large and small ascospores in asci; no asci contain all large or all small spores of the extreme dimensions. The present estimate of ascospore size in *E. albus* differs considerably from Eidam's original measurements (5·2–5·5 μ), but no further comparison is possible since Eidam's material did not survive. It is proposed that the present estimate be taken as the ascospore size of *E. albus* since there is good agreement between the separate estimates for the five new isolates of this species.

Size of asci, thallospores, and hyphae. Eidam (1883) gave the diameter of the asci of *E. albus* as 12·5–13 μ, but in the present isolates, where the ascospores are larger, the ascus diameter is 15 μ (range 12–19 μ), measured when the ascospores are fully formed (i.e. with a double wall) but while the ascus wall is still intact.

Thallospore size in the present material cannot be compared with any corresponding estimate in the original description. The average diameter of thallospores in *E. albus* is 10 μ (range 7–17 μ) with 7, 8, and 9 μ as most frequent.

The hyphae in *E. albus* are 3–4 μ in diameter.

The following diagnosis based on the characters of the five new isolates is proposed:

Eremascus albus: Colony regular, convex; white, ageing to primrose yellow; hyphae septate 3–4 μ diameter. Thallospores with thickened walls, 10 μ diameter, terminal or intercalary. Asci spherical, 15 μ diameter, naked and solitary on pairs of coiled gametangia; 1–4 gametangial coils; several gametangia may arise on the same part of a hypha. Ascospores almost spherical, 7·7 × 6·5 μ; eight per ascus, liberated by dissolution of the ascus wall.

Eremascus fertilis. Stoppel (1907) laid no special emphasis on the cultural characters of her isolate, recording only that a 15 per cent. apple-jelly medium gave the best growth. The present cultures (A, B, and C) on 2 per cent. malt agar produce a slow-growing corrugated colony. Lateral spread is very slow and the hyphae are composed of irregular cells. Pl. IV, *i*, *a* shows a colony at 25° C. on malt agar, 2 months old. On malt–40 per cent. sucrose agar a flat white colony with sparse aerial mycelium develops (Pl. IV, *i*, *c*), which is quite different from the dense corrugated colony produced on malt–40 per cent. sucrose–0·5 per cent. Difco yeast agar (Pl. IV, *i*, *b*). Such colonies are white at first but gradually change to amber yellow (50. 5/3; no Ridgway equivalent). In colonies on the sucrose agars the hyphae are typical and contrast sharply with the short distorted hyphae produced on malt agar.

TABLE II

Average Ascospore Size in Eremascus fertilis

Culture.	Substrate.	Ascospore size (μ).
A	M 40	6·3 × 3·8
B	M 40	6·9 × 3·8
	M	5·6 × 3·1
C	Jam	6·3 × 3·1
	M 40	5·6 × 3·1
	M	5·6 × 3·8

(For explanation of M and M 40 see Table I.)

Ascospore size. The average dimensions are $6{\cdot}1 \times 3{\cdot}5\,\mu$, with $6{\cdot}3 \times 3{\cdot}8\,\mu$ as the most frequent class. The range lies between $3{\cdot}8$–$10{\cdot}0\,\mu$ in length and $2{\cdot}5$–$5{\cdot}0\,\mu$ in breadth. The ascospores of the present cultures are larger than those of the type species (Stoppel, 1907, $5{\cdot}2 \times 3{\cdot}0\,\mu$), but the discrepancy is small and no useful purpose would be served by designating these cultures as different varieties, since comparison with the original ones is impossible.

V. CYTOLOGICAL STUDIES

No cytological studies were carried out on *E. albus* by Eidam (1883). Stoppel (1907) and Guilliermond (1909) investigated the cytology of *E. fertilis*, but they were unable to make out chromosomes in their material.

Materials and methods. Germ-tube material was obtained by adapting a cellophane strip technique used by Robinow (1942) in his studies of bacterial cytology. Strips of permeable cellophane (British Cellophane Company, No. 300) were sterilized by autoclaving in a tube of water and then placed on malt-sucrose agar in a Petri dish after removing the excess of water between sheets of sterile blotting-paper. Ascospores were dusted on to the cellophane surface and then incubated. The strip bearing the germ-tubes was then lifted off and placed in the fixative and stained. Material of the asci was obtained in essentially the same way, incubated at 25° C. over a 16 per cent. saline solution until suitable stages of ascus formation developed. This required 6 days for *E. albus* and 7 days for *E. fertilis.* Development at 90 per cent. R.H. (approximately the value over 16 per cent. saline solution in a Petri dish) is faster than in a dish of malt–40 per cent. sucrose agar where the relative humidity is about 98 per cent. Relative humidity is a very important factor controlling the growth of *E. albus* and will be discussed in detail in a second paper. Use of the method outlined above saves considerable time in producing asci for cytological studies.

Acetocarmine and similar stain-fixatives applied with or without prior fixation of germ-tubes in acetic alcohol (1 : 3) were not successful. Aqueous fixation with La Cour's 2BE fluid was satisfactory, and if the germ-tubes were less than 4 days old they did not float off from the cellophane strip during fixation and washing. Fixed germ-tubes were stained with iron alum-haematoxylin according to the schedule of Darlington and La Cour (1942). This technique revealed the nuclei as light circular areas each with a deeply staining nucleolus. No details of chromosomes or spindles were visible, and variations in times of fixation, mordanting, and differentiation gave no improvement.

The Feulgen technique was applied to germ-tubes and asci without success.

Treatment overnight in acetic alcohol gave good fixation of the asci, but since acetic alcohol is not a satisfactory fixative for subsequent haematoxylin treatment, material fixed in this way was mordanted in a chromic acid solution (e.g. La Cour's 2BE fluid without the addition of osmic acid) for 30 minutes, followed by thorough washing prior to staining.

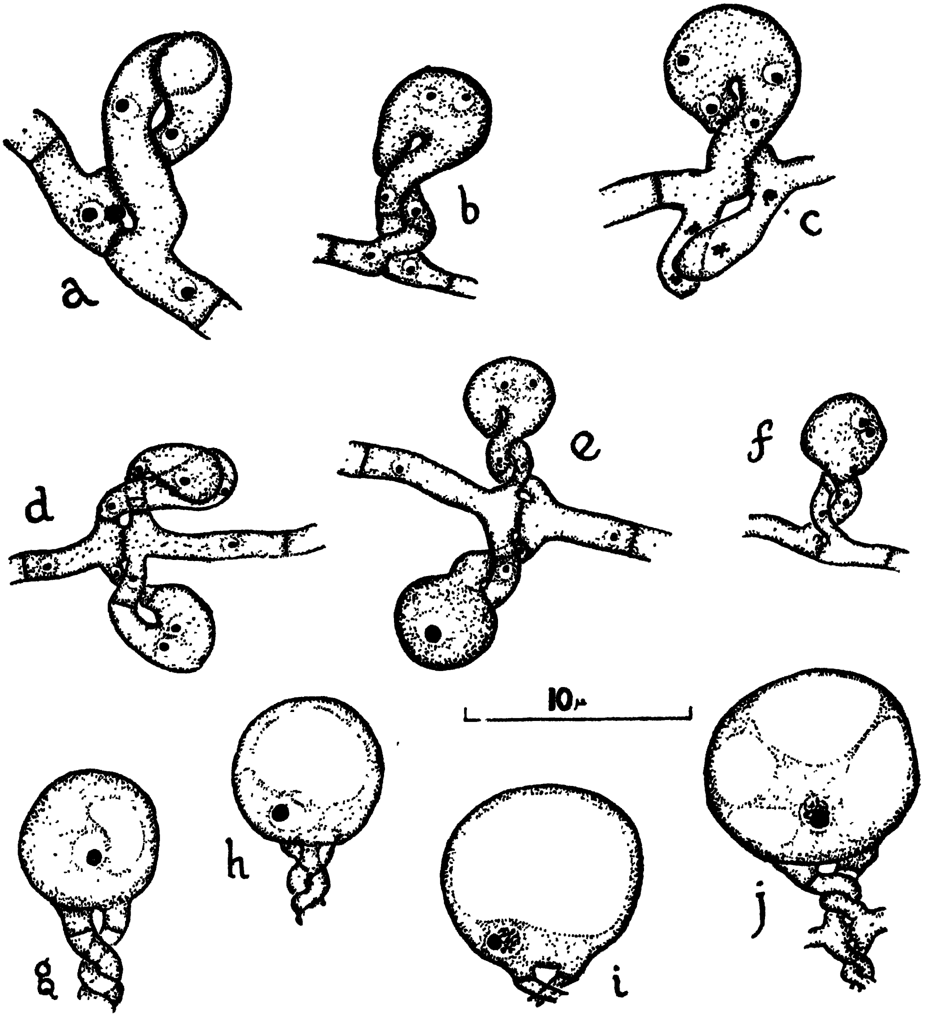
Haematoxylin preparations gave a good picture of resting nuclei during ascus formation, but details of chromosomes during nuclear division were obscure. The modified acetocarmine squash method described below gave much clearer pictures of the chromosomes but left the resting nuclei unstained. Each method, therefore, was used to supplement the other in studying the nuclei during ascus development.

Studies of the chromosomes of *Neurospora crassa* (McClintock, 1945) and *N. tetrasperma* (Cutter, 1946 *a*, *b*), using the acetocarmine method without modification, have lately appeared. Material of *E. albus* was subjected to similar treatment without success. Fincham (1949) working with *Neurospora crassa* and *N. sitophila* likewise found that the methods of McClintock and Cutter did not suit his material unless the following modification, suggested by Godward (1948) for algae, was used. Material of *E. albus* (fixed in acetic alcohol) was mordanted in 4 per cent. iron alum solution for periods of 30 seconds, 1, 3, 5, 10, and 30 minutes, and subjected to the usual acetocarmine squash method after washing out the excess iron alum. This technique gave satisfactory chromosome preparations, the best results being obtained after 5 minutes' mordanting or less. Mordanting for 10 minutes or more caused too deep staining of the cytoplasm. In *E. albus* only the dividing nuclei are properly stained; the resting nucleus is poorly differentiated, a nucleolus is rarely distinguishable, and no details of the prophase of mitosis or meiosis can be seen.

Results

Eremascus albus. The ascospores are uninucleate when germination begins; as the spore pushes out the vesicle from which the germ-tubes arise the nucleus divides. Further divisions follow rapidly and as the germ-tubes grow the spore case becomes empty. The apices of the germ-tubes are multinucleate; as many as twenty nuclei were counted in one long terminal cell. After 3–4 days the germ-tubes have developed into segmented hyphae with uninucleate cells, which is the predominant adult condition, although multinucleate cells can be found occasionally. Division figures in the germ-tubes and hyphae were too small for chromosome counts to be made.

The gametangia arise from adjacent pairs of uninucleate cells. As the parent cell forms the outgrowth which will become the gametangium its nucleus divides, and one of the daughter nuclei passes into the outgrowth leaving the other within the parent cell (Text-fig. 10, *a*, *b*). A cross-wall is laid down (Text-fig. 10, *b*), and the parent cell may then give rise, by a similar process, to further gametangia close to the first (Text-fig. 10, *c*, *d*, *e*). Once the gametangium is cut off from the parent cell (Text-fig. 10, *b*), or before this happens (Text-fig. 10, *c*), its nucleus divides once and the upper one passes to the tip of the gametangium where it is destined to act as a gamete. This division takes place prior to fusion of the gametangia (Text-fig. 10, *d*); after this fusion has taken place the gamete nuclei (which have no special characters making them visibly different from each other or other nuclei) meet and fuse (Text-fig. 10, *e*, *f*). There is no synchronization between the development of a number of gametangial pairs from the same parent cells. Thus Text-fig. 10, *e* shows fusion of the lower gametangia whilst the two gamete nuclei are still approaching each other in the upper pair. The gamete nuclei and the fusion nucleus arising from them may be cut off from the lower part of the gametangium before or after fusion has occurred.



TEXT-FIG. 10. *E. albus*: nuclear behaviour during ascus formation. (Iron-alum haematoxylin stain.)

Swelling of the fused tips of the gametangia to form the ascus takes place after the nuclear fusion; such swelling is correlated with the appearance of a vacuole alongside the fusion nucleus which is generally at the base of the ascus and to one side (Text-fig. 10, *g*, *h*, *i*). This vacuole disappears before the ascospores are cut out (Text-fig. 10, *j*). During vacuolation and devacuolation the fusion nucleus passes into the prophase of the ensuing meiosis (Text-fig. 10, *i*, *j*). The nucleus undergoes three divisions (Pl. IV, *k*, *l*, *m*) and since only one nuclear fusion occurs there can be no question of brachymeiosis. The ascospores differentiate around the eight nuclei and are formed in the manner typical of the Ascomycetes.

Great effort was made to analyse the details of the nuclear divisions in the ascus, but no completely satisfactory preparations were obtained from which unassailable information concerning chromosome number and structure could be derived. Pl. IV, *j* shows metaphase I during meiosis.

Eremascus fertilis. The situation was more difficult in *E. fertilis* where the asci and nuclei are even smaller than in *E. albus*. Stoppel (1907) and Guilliermond (1909) had no success in seeing chromosomes in their material. Using the technique described above, acetocarmine preparations of the chromosomes during ascus division were obtained, but it proved impossible to count the chromosomes in this species as they were too small.

VI. Discussion

A normal process of isogamous fusion leading to ascus-formation in *E. albus* has been described, together with variations towards anisogamy and fluctuations in ascospore number per ascus. These facts may be adduced to support a derivation of the Ascomycetes from the Phycomycetes in which the Endomycetaceae and forms like *Ascoidea* and *Dipodascus* resemble transition types between the two groups. Such a derivation has been postulated by many mycologists, notably de Bary (1884), Atkinson (1915), and Gäumann and Dodge (1928), who stated that 'the step from the Zygomycetes to the Hemiasci (i.e. Hemiascomycetes) would consist in the differentiation of the germ sporangium to an ascus conditioned by the privileging of sexual nuclei, while the structure of the sexual organs themselves and the place of caryogamy remains unaltered'.

Ascoidea rubescens (Walker, 1931, 1935) is a form showing both phycomycete and ascomycete characters and is probably a specialized line away from the main path of evolution, since Walker could find no evidence of sexual fusion at any stage of the life-history. The spore sac of *A. rubescens* is therefore best regarded as an apogamously developed gametangium. *Dipodascus albidus* (Lagerheim, 1892; Juel, 1902, 1921) is closer to the suggested evolutionary line, and has the phycomycete character of a multispored 'sporangium' together with the predominantly ascomycete characters of free cell-formation of the spores and direct development of the fused gametangia to produce the 'ascus'. Biggs (1937) reported another species, *D. uninucleatus*, in which the cells of the hyphae and gametangium are uninucleate, bringing this species indubitably into the ascomycete group and indicating the form of the probable transition types between *D. albidus* and members of the Endomycetaceae.

The line of evolution suggested above requires a reduction in the spore number in ancestral multispored forms resembling *D. albidus*, culminating in the eight or four spores per ascus of *Eremascus* and *Endomyces*. Atkinson (1915) remarked that in older cultures of *D. albidus* the asci became smaller and the spores less numerous, suggesting a tendency towards the reduced number in *Eremascus*. We have seen that *E. albus* (and also *E. fertilis*) can produce more than eight spores per ascus (Text-fig. 6, *b*) indicating that this character is not fixed; the possibility of some ancestral spore number greater than eight is thus not precluded. Morphological resemblances between the sexual processes in *Dipodascus albidus* and *E. albus* have also been noted, which support the idea of relationship between *Dipodascus* and members of the Endomycetaceae. The variations in the gametangia of *E. albus* also indicate a way in which the antheridia and ascogonia of higher Ascomycetes may have evolved from an isogamous ancestry.

The Endomycetales comprise very simple forms, and it is necessary to decide whether this simplicity is primitive or due to reduction caused by the specialized habitats in which many of these fungi live; thus, the Saccharomycetaceae are generally accepted as reduced forms specialized for living in sugary habitats. *E. albus* cannot grow in saturated conditions and its habitat range is therefore limited in comparison with most fungi. None of the Endomycetaceae show much development of conidia or special mechanisms for ascospore dispersal, and their ascospores are often resistant and long-lived. Thus, ascospores of *E. albus* will germinate successfully 3 years after their formation, and *Endomyces fibuliger* produces mycelial buds which can survive long periods at 55° C. (Gäumann and Dodge, 1928, p. 141). These moulds stand in sharp contrast to the ubiquitous Aspergilli and Penicillia with their highly developed methods of spore production. The Endomycetales comprise many forms living in special habitats of spasmodic occurrence (e.g. slime fluxes) and have no well-developed method of spore dispersal. The spores themselves for the most part result from a sexual process or some modification of this; there is little development of asexual spores.

The members of the Endomycetaceae are homothallic and the tendency towards parthenogenesis is probably connected with the loss in significance of the sexual process through the fixed occurrence of homothallism. Heterothallism appears to be a necessary condition for the occurrence of progressive evolution in certain groups of Fungi, and perhaps it is possible to correlate the prevalent homothallism of the Endomycetaceae with an adaptation to special habitats where outbreeding mechanisms might not be helpful for continued survival. Alternatively, the occurrence of homothallism may have led to fixity of habitat.

The Endomycetaceae are therefore regarded as simple types in which further simplification due to reduction is found, e.g. the breakdown of the sexual process leading to parthenogenesis. Some, particularly *Eremascus*, still retain primitive characters such as a sexual process involving fusion of equal gametangia with direct development into a single naked ascus. *Eremascus* is regarded as closely resembling transitional forms on the suggested line of development of the Ascomycetes from the advanced Phycomycetes (e.g. *Endogone*) through intermediate stages indicated by the Hemiascomycetes (e.g. *Dipodascus*).

VII. SUMMARY

1. The Ascomycete *Eremascus albus* Eidam, lost since 1883, has been found again. Five new isolates from mustard powder, mincemeat, and jam have been obtained.
2. The original description has been generally confirmed and amplified.
3. The species is homothallic. Variations of the normal sexual process, ascus shape, and ascospore number are recorded and discussed.
4. The formation of ascospores inside enlarged hyphal cells and the occurrence of thallospores are described for the first time.
5. The taxonomic characters of the genus *Eremascus* are discussed, and cultural characters distinguishing the two species are described.
6. Ascospore size in the present isolates of *E. albus* is 7·7 × 6·5 μ; this differs appreciably from the size of the ascospores in Eidam's original description.
7. Ascospore size in *E. fertilis* is 6·1 × 3·5 μ; this differs slightly from Stoppel's original figure.
8. The cytology of *E. albus* during germination, gametangium- and ascus-formation is described for the first time, but the chromosome number could not be definitely determined.
9. The status of the Endomycetales is discussed.

VIII. ACKNOWLEDGEMENTS

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EXPLANATION OF PLATES

Illustrating C. E. Harrold's article 'Studies in the Genus *Eremascus*, I. The Rediscovery of *Eremascus albus* Eidam and Some New Observations concerning its Life-history and Cytology.'

j, k, l, m: iron-alum acetocarmine squash preparations. *a–f*, ×700; *g*, ×500; *h, i*, ×1; *j–m*, ×2,500.

a. E. albus: Germination of ascospores. Note small vesicle out of which the germ-tubes grow.

b. E. albus: Gametangia coiling.

c. E. albus: Fusion of coiled gametangia.

d. E. albus: Fusion of gametangia, swelling of ascus, and delimitation of ascospores.

e. E. albus: Terminal thallospores.

f. E. albus: Thallospores on germ-tubes.

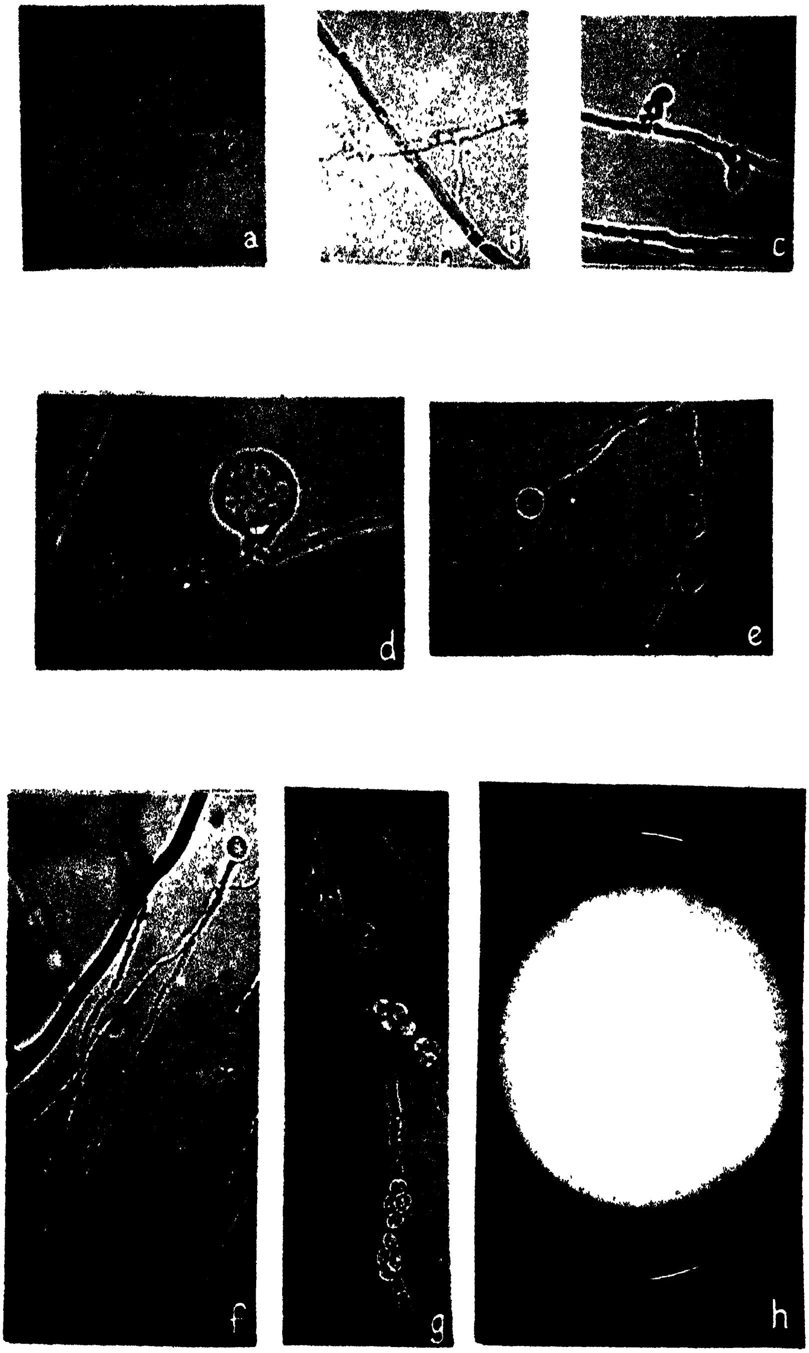
g. E. albus: Ascospore development in hyphae.

h. E. albus: Colony on malt–40% sucrose–0·5% Difco yeast agar.

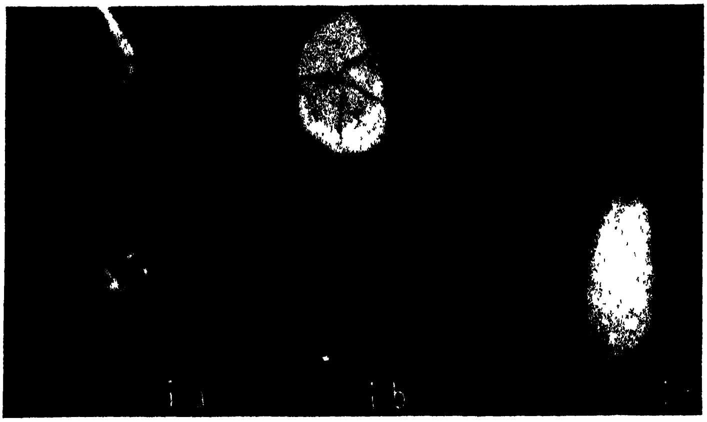
i. E. fertilis: Colonies on (*a*): malt agar; (*b*): malt–40% sucrose–0·5% Difco yeast agar; (*c*): malt–40% sucrose agar.

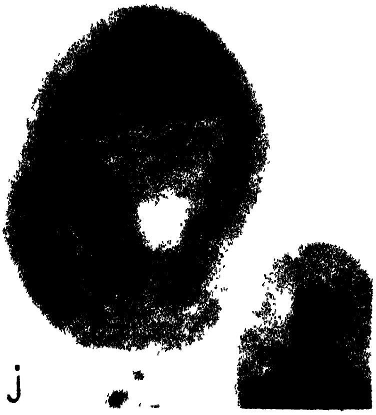
j. E. albus: Metaphase I of meiosis in the ascus.

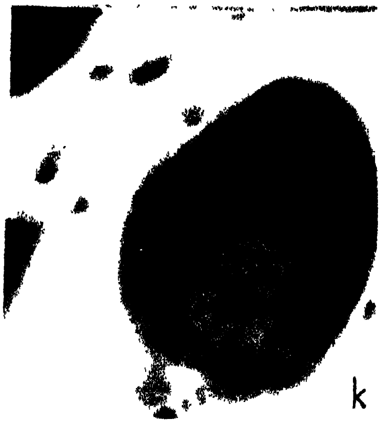
k, l, m. E. albus: Nuclear divisions in the ascus showing successive formation of 2, 4, and 8 nuclei.

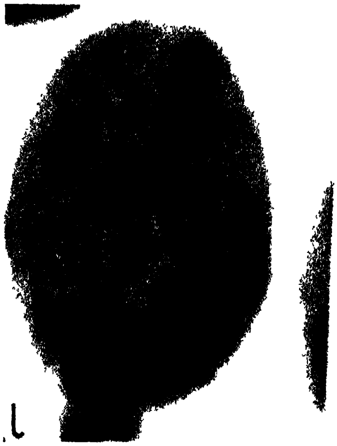


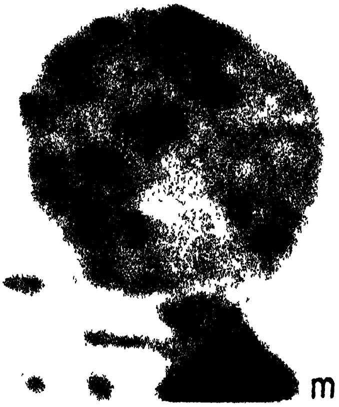
C. E. HARROLD—*Eremascus*











C. E. HARROLD—*Eremascus*

Wilt of Cacao Fruits (*Theobroma Cacao*)

V. Seasonal Variation in Potassium, Nitrogen, Phosphorus, and Calcium of the Bark and Wood of the Cacao Tree

BY

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With two Figures in the Text

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INTRODUCTION

IN the previous paper of this series (Humphries, 1947) the results of an experiment were described in which the seasonal changes in water content and carbohydrate reserves (starch, sucrose, reducing sugars, alcohol-soluble material) as well as non-carbohydrate alcohol-soluble material at three levels in the bark and wood of the cacao tree were followed during a period of 14 months. It was established that significant changes in most of these constituents occurred in response to vegetative changes and to crop production. No evidence was obtained that the available carbohydrate supply limited the size of the crop and it appeared probable that the supply of one or other of the mineral elements was the critical factor.

At the same time that the samples for carbohydrate estimations were taken, subsamples were oven-dried for subsequent mineral analysis. It is the purpose of the present paper to discuss the seasonal changes in certain elements and at the same time to see how these are related to the carbohydrate changes. The data have been subjected to the same scheme of statistical analysis as in the previous paper to establish whether significant changes between seasons and levels occur.

SEASONAL CHANGES IN THE WOOD

The mean values of the mineral elements present in the wood are shown in Table I together with the degree of significance between seasons and between

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levels. There is a highly significant seasonal variation in *potassium.* The difference between levels is also significant, and as the greatest amount occurs at the 1-ft. level there is a negative gradient of this element in the wood. The seasonal changes of potassium in the wood are positively correlated with changes of this element in the bark. The potassium figures for wood and bark are shown in Appendix, Table I. In the case of *phosphorus* there is also a highly significant difference between seasons and between levels and a consistently negative gradient (see Appendix, Table IV). Total *nitrogen* shows no significant seasonal variation and no significant difference between levels (Appendix, Table III). There is no seasonal change in *calcium*, but a negative gradient exists between the levels investigated.

TABLE I

Mean Content of Mineral Constituents in Bark and Wood (g. per 100 g. Water), Degree of Significance of Difference between Levels and Seasons, and Correlation between Like Constituents of Bark and Wood

	BARK.			WOOD.					
		Degree of significance (*P*) of difference between			Degree of significance (*P*) of difference between		Correlation between bark and wood.	Direction of gradient.	
	Mean.	Levels.	Seasons.	Mean.	Levels.	Seasons.	Seasons.	Bark.	Wood.
Potassium	0·438 ± 0·009	0·05	0·001	0·453 ± 0·005	0·001	0·001	+0·755 (*P* - 0·01)	− ve 1 ft. to 6 ft. + ve 6 ft. to 12 ft.	−ve
Calcium	3·688 ± 0·060	0·001	0·05	0·894 ± 0·021	0·01	Not sig.	+0·338 not sig.	−ve	−ve
Phosphorus	0·031 ± 0·0004	Not sig.	0·05	0·047 ± 0·0010	0·001	0·01	+0·509 not sig.	0	−ve
Nitrogen	0·382 ± 0·004	0·01	0·05	0·372 ± 0·008	Not sig.	Not sig.	+0·471 not sig.	−ve 1 ft. to 6 ft. +ve 6 ft. to 12 ft.	0

The data were examined to see if they revealed any marked trend during the dry season and especially whether there was any indication of restricted uptake during this period. It may be inquired what conditions, according to current conceptions of mineral transport, would exist in the tree if the uptake of minerals by the roots is reduced during the dry season. It is generally accepted that the wood is the upward channel of transport of mineral substances and assuming that the rate of uptake from the soil is fairly constant, as might be expected under the fairly uniform soil and atmospheric environments which exist during the wet season in Trinidad, the total gradient in the wood of any particular element should be reasonably constant (assuming that the static or structural gradient of this element is also constant). This assumption is supported in the present case by the data in Fig. 1, where the gradients of calcium, potassium, phosphorus, and total nitrogen in the wood between the 1-ft. and 12-ft. levels are plotted. It will be noticed that there are negative gradients of calcium, potassium, and phosphorus, and moreover that these gradients do not vary much during the period June–December, that is, during the wet season. There is, however, a diminution in the gradient during the dry season, but the extent of this decrease varies with the different elements. In the case of calcium there is a steady decrease in gradient from the onset of the dry season (January) until the gradient has virtually disappeared after 5 months of dry weather. During the ensuing wet season the gradient quickly becomes established again. With potassium there is not such a marked change of gradient at the beginning of the dry season but it falls during the last month to a low value. There appears to be no change of the gradient of phosphorus during the whole period of the experiment and therefore no dry-season effect. The differences in gradient changes of these three elements seem best interpreted, not as an effect of drought on the differential uptake of these elements, but as connected with differences in redistribution

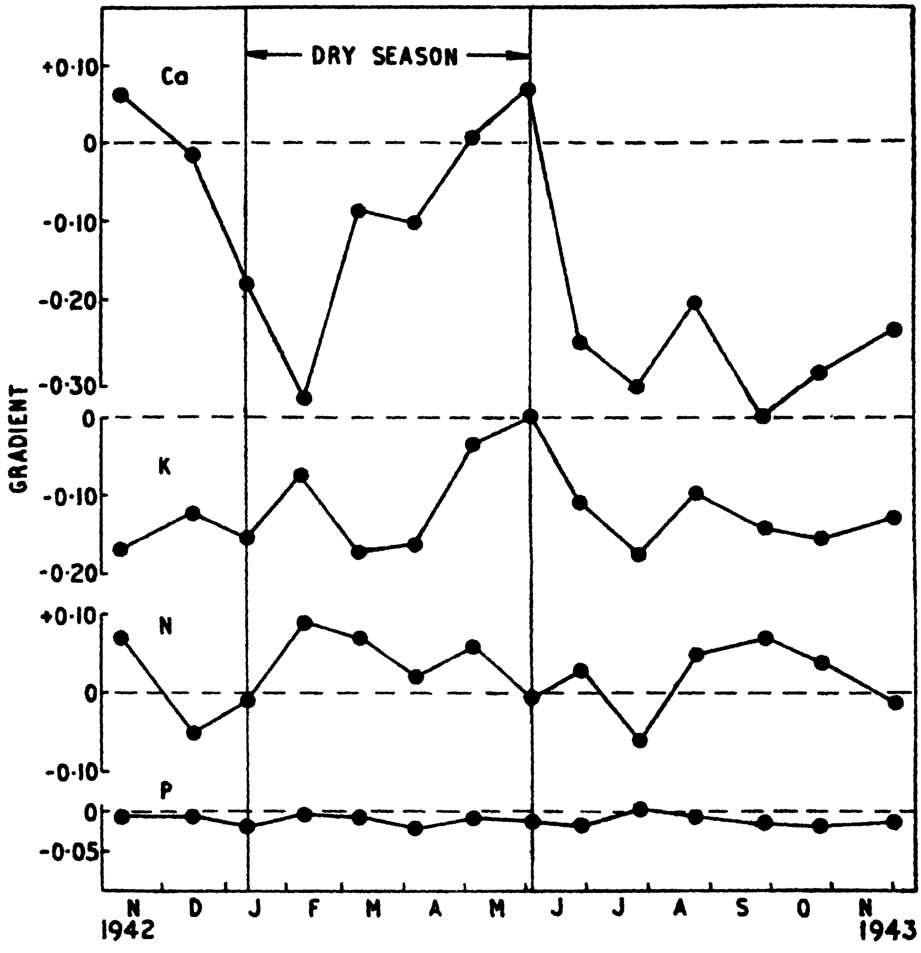

FIG. 1. Seasonal changes in gradients of calcium, potassium, total nitrogen, and phosphorus, g. per 100 g. water, in the wood between the 1-ft. and 12-ft. levels.

of the elements within the plants when the supply from the soil has been reduced to a low level. The work of Mason and Maskell has shown that calcium is not phloem-mobile and is not re-exported from tissues which it reaches via the transpiration stream. In other words, there is little possibility of redistribution of calcium, and hence this element would be expected to be the first to show a diminished gradient when uptake from the soil is restricted. In the case of potassium and phosphorus, however, Mason and Maskell have shown that in the cotton plant these elements are readily re-exported via the phloem and can leak back into the xylem vessels. If this occurs in the cacao tree in the dry season, a gradient of these elements could readily be maintained by transport from the bark into the wood at the same level even though there is no net uptake from the soil. That potassium does show a decrease in gradient at the end of the dry season is probably connected with the fact that potassium tended to be deficient in the experimental trees, as will be shown later from other evidence. Thus the observations appear to be in accord with the supposition that there is a progressively diminishing uptake from the soil during the dry season and that by the end of the dry season this uptake has almost if not entirely ceased.

It is extremely likely that as the dry season progresses the transpiration rate increases, but unfortunately there is no direct evidence on this point. In the dry season there are several environmental factors which would tend to increase the transpiration rate. The cacao-shade trees (*Erythrina* spp.) lose their leaves during this season so that insolation is greatly increased. The atmospheric humidity is low and the amount and velocity of the wind increases. There is invariably a big vegetative flush (and sometimes two) at the height of the dry season in response to the higher temperatures (Humphries, 1944 *a*). All these factors would tend to increase the transpiration rate in spite of the diminished uptake from the soil. The fall in water content of the wood during the dry season is probably accounted for by a combination of these two factors. In the foregoing argument only the total gradients have been considered and it is not known what fraction of the total amount of each element is actually present in the transpiration current. If, however, the calcium, for instance, of the transpiration current ceases to be renewed, a decrease in calcium gradient would be expected. If the calcium of the transpiration stream is only a small fraction of the total calcium, then the predominating gradient of structural and accumulated calcium would show little change in response to drought. Since there is a response with the gradients decreasing as the season progresses, it suggests that either the total calcium is made up of quite a large proportion of transpirational calcium or that there is a redistribution of calcium in the wood during the dry season. This latter alternative is in conflict with the generally accepted idea that calcium is not readily remobilized and hence the former alternative appears to be the case.

The data for the calcium gradients plotted in Fig. 1 have been examined statistically to see if the dry-season gradients are in fact significantly lower than those of the wet season. It is found that the average gradient over the whole of the dry season is not significantly different from the average wet-season gradient, but it is not to be expected that the full effect of drought on uptake from the soil will be immediately evident as the drying out of the soil is a gradual process.[1]

The average value of the gradient in the last three months of the dry season, viz. —0·01 g. Ca, is significantly lower than the average gradient during the remainder of the period under investigation, viz. —0·20 g. Ca. These gradients expressed as g. per 100 g. water possibly exaggerate the magnitude of the difference between seasons, since there is a progressive decrease in water content of the wood during the dry season (Humphries, 1947), so that the gradients calculated on a dry-weight basis have also been examined. These show a similar effect and the gradients in the last two months of the dry season are significantly lower than in the rest of the period.

SEASONAL CHANGES IN THE BARK

Potassium

The potassium content of the bark is of the same order of magnitude as that of the wood (Table I and Appendix, Table I), but slightly lower in the bark at the 1-ft. and 6-ft. levels and slightly higher at 12 ft. Seasonal changes of total potassium in the bark are significant ($P = 0{\cdot}001$) and the levels differences are also significant. It may be seen from the data in Appendix, Table I, that the concentration of potassium is usually highest at the 12-ft. level and lowest at the 6-ft. level. The seasonal drifts in potassium contents of the three levels are not strictly parallel to one another, so that differences in *gradients* between the levels occur, and it is proposed to discuss the data in terms of gradients rather than consider the seasonal changes in concentration at any particular level. As mentioned above, the concentration of potassium in the bark is usually highest at the 12-ft. level and lowest at the 6-ft. level, so that there is a negative gradient between 1 ft. and 6 ft. and a positive gradient between 6 ft. and 12 ft. This could be explained by assuming that there is a negative structural and storage gradient which is relatively much steeper between 1 ft. and 6 ft. than between 6 ft. and 12 ft. This appears reasonable since the 1-ft. region is ontogenetically older than the 12-ft. region. It is further assumed that the storage gradient in the lower region completely masks the positive dynamic gradient. Between the 6-ft. and 12-ft. levels, however, the gradient is positive and here the dynamic gradient predominates over the relatively small storage gradient. As a consequence it may be assumed that the seasonal changes in gradient in the upper region are in the main a reflection of the changes in the positive dynamic gradient, while the changes in gradient in the lower region are due to changes in the storage gradient.

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[1] Data on the change in soil moisture (0–12 in.) during the dry season were obtained for the same estate and soil type in a previous experiment (Humphries, 1944 *b*). There was an almost linear fall in gross soil moisture from about 25 per cent. in January to 16 per cent. in May at the end of the dry season.

The month to month changes in the total potassium gradient in the two regions are plotted in Fig. 2.

It was postulated by Mason and Maskell (1931) that so long as the supply of an element is relatively abundant, the cells of the bark continue to store the surplus that is not required for immediate growth. A negative storage gradient would then exist in the bark. If the supply were restricted, there might be no appreciable storage and the vertical gradients in the bark might then be positive, representing the positive dynamic gradient with its transport head in the foliage region. The present data appear to bear out this contention. As will be shown later, certain of the experimental trees are lower (and probably deficient) in potassium. It would be expected that the trees more deficient in potassium would tend to have greater positive gradients between

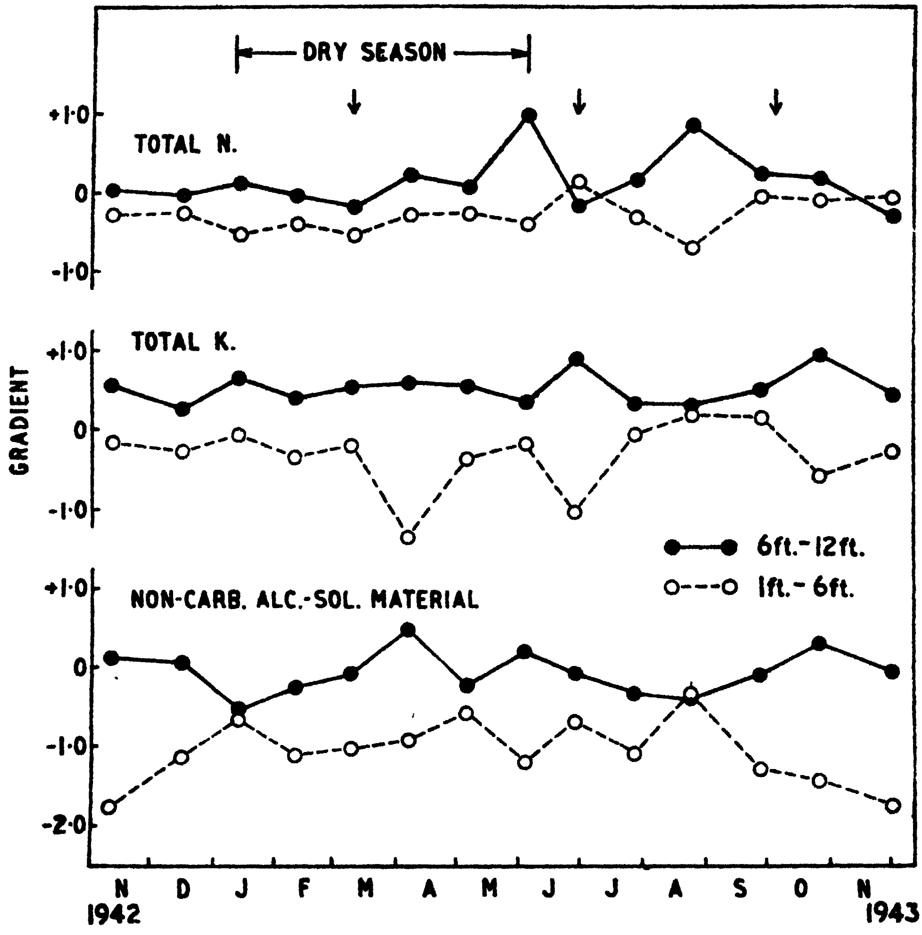


FIG. 2. Seasonal changes in gradient of total nitrogen, potassium, and non-carbohydrate alcohol-soluble material in the 1-ft. to 6-ft. region and the 6-ft. to 12-ft. region in the bark. The downward-pointing arrows indicate the times of occurrence of vegetative flushes.

6 ft. and 12 ft. than the others. In Table II are presented the gradients in this region for the two sets of trees. It will be noted that in the B series the gradient is greater than in the A series nine times out of the fourteen sampling occasions. The gradient in the 1-ft. to 6-ft. region (considered to be mainly due to storage potassium), on the other hand, shows the opposite effect. Here the gradient is greater in the A series eleven times out of the fourteen occasions. This is interpreted as indicating that the storage potassium in the deficient trees falls off more rapidly with increasing height than in the other trees—a conclusion which is in accord with expectation.

TABLE II

Gradients of Total Potassium (g. per 100 g. Water) in Two Regions of the Bark in the Two Sets of Experimental Trees

Date of sampling.	1 ft.–6 ft.		6 ft.–12 ft.	
	A	B	A	B
Nov. 10	−0·42	+0·08	+0·27	+0·83
Dec. 16	−0·28	−0·27	+1·00	−0·50
Jan. 12	+0·73	−0·88	+0·66	+0·61
Feb. 9	0·21	−0·55	+0·61	+0·15
Mar. 9	−0·03	−0·41	+0·90	+0·11
Apr. 6	−2·84	+0·11	+0·50	−0·64
May 5	+0·05	−0·80	+0·30	+0·78
June 3	+0·27	−0·66	−0·32	+0·97
June 29	−0·69	−1·42	+0·53	+1·23
July 27	+0·44	0·60	−0·03	+0·65
Aug. 24	+0·72	0·38	−0·41	+0·16
Sept. 28	+0·59	−0·34	0·04	+0·93
Oct. 27	−0·43	−0·76	+0·84	+0·99
Dec. 1	−0·70	−0·13	+0·04	+0·77

It would be anticipated that at time of greatest stress or of greatest demand for an element there would be a tendency for positive (dynamic) gradients to become evident or for existing positive gradients to increase. From the gradients of total potassium between the 6-ft. and 12-ft. levels plotted in Fig. 2 it will be noticed that there are five occasions when the positive gradients reach high values: in November, at the beginning of the experimental period when there is a fairly large crop on the trees; in January; in April, during the dry season following a new flush of leaves; in July, at the time of occurrence of a flush; and in October, when there is a large crop on the trees and immediately after a flush has occurred.

Nitrogen

Only analyses of total nitrogen are available and these do not furnish much information about the true dynamic and storage gradients at the different seasons, but as the changes in total nitrogen give indications of a definite trend they will be briefly discussed. The summary in Table I indicates that there is a significant seasonal change in total nitrogen of the bark and that the difference between levels is also significant. The average content of total nitrogen in both samples is lowest at the 6-ft. level (see Appendix, Table III), and there is therefore a change from a negative gradient between 1 ft. and 6 ft. to a positive gradient between 6 ft. and 12 ft. as in the case of potassium. This again appears to be a case of the storage and structural gradient predominating in the lower region and a dynamic gradient predominating in the upper region. No evidence was obtained from the experiment that nitrogen was limiting crop production in any of the experimental trees, but nevertheless it might be expected that a change of gradient would occur in response to increased demand for nitrogen by the crop or foliage or when net uptake from the soil is restricted. It would then be expected that a negative gradient would become positive or that an existing positive gradient would be increased, as demonstrated in the case of potassium. The changes in gradient of total nitrogen with time in the two regions are shown in Fig. 2. In the 1-ft. to 6-ft. region the gradients are all negative with one exception at the end of June when a slight positive gradient exists. The gradients in the 6-ft. to 12-ft. region, on the other hand, are predominantly positive. The gradient in the 6-ft. to 12-ft. region is only slight in the first 5 months of the experiment but increases at the end of the dry season to a maximum (June). Another maximum positive gradient occurs in August.

It is of interest in connexion with changes in nitrogenous reserves to consider further the non-carbohydrate alcohol-soluble material of the bark reported in the preceding paper of this series. This fraction consists in part of soluble nitrogenous substances some of which will be concerned in movement in the bark. Hence changes in this fraction will, to a certain extent, reflect changes both of the storage and dynamic nitrogen. The seasonal drift of this fraction in the two regions is shown in Fig. 2. Between 1 ft. and 6 ft. the gradients are consistently negative as in the case of total nitrogen. At the beginning of the experimental period (when there is a fairly large crop on the tree) the gradient has a large negative value which gradually decreases. The gradient becomes increasingly negative again at the end of the experimental period when a developing crop and a recent flush make demands on the reserves. The gradients in the upper region are both positive and negative. The largest positive gradients occur at the beginning of the experimental period, in April and June (dry-season months) and again in November. All these times correspond with periods of increased utilization of reserves. The first and last are periods of crop demand, while April and June are dry-season months when uptake from the soil is reduced to a minimum and when renewed vegetative growth is occurring.

Phosphorus

The content of total phosphorus in the bark is about half the concentration in the wood (Appendix, Table IV). There is a significant seasonal change but no difference between levels. There does not appear therefore to be any gradient of total phosphorus, and although doubtless some movement of phosphorus takes place in the bark it is masked by the remaining structural phosphorus. The phosphorus of the bark is not correlated with the phosphorus of the wood at any level.

Calcium

The calcium content of the bark is about four times that of the wood (Appendix, Table II) and the gradient is consistently negative throughout the length of trunk examined. The seasons correlation between the calcium of the wood and that of the bark at 1 ft. is high ($r = +0{\cdot}707$, $P = 0{\cdot}01$), which probably indicates considerable cross-leakage of calcium from the vessels of the wood into the bark. No significant correlations were found at other levels.

THE INFLUENCE OF CROP ON THE CARBOHYDRATE AND MINERAL RESERVES AND THE RELATIONSHIPS BETWEEN POTASSIUM, PHOSPHORUS, AND CARBOHYDRATES

In the previous paper of this series, using total dry weight as an estimate of crop size, it was possible by means of an analysis of covariance to assess the effect of crop demand on the carbohydrate reserves of the bark and wood. The data permitted an estimate of effects due to seasonal changes (13 degrees of freedom) and residual effect (14 degrees of freedom) at any of the three levels investigated (Humphries, 1947, p. 220). In the case of sucrose, for example, there was a high negative seasonal correlation with crop, pointing to the depletion of sucrose reserves as crop increased. In the case of the residual component also the correlation was negative and significant, indicating that a higher sucrose content was associated with a smaller crop, and it was concluded that carbohydrate (sucrose) was not limiting crop size but that some other factor was responsible.

A similar set of analyses have been carried out in the case of the mineral components, and the correlations between crop and potassium are presented in Table III. The correlations between crop and nitrogen and crop and phosphorus prove to be non-significant. In the case of potassium, in the bark, the seasons correlation has a fairly high negative value at all levels and just fails to reach significance at 6 ft. This indicates a drain on the potassium reserves of the tree with increasing crop. The residual correlations, however, are all *positive* and reach significance at 1 ft. and 6 ft. and also when the total of the three levels are considered (i.e. equivalent to a composite sample of the

TABLE III

Correlation Coefficient between Crop and Potassium Content

	Bark.		Wood.	
	Seasons.	Residual.	Seasons.	Residual.
1 ft. . .	−0·431	*+0·516*	0·508	−0·176
6 ft. . .	−0·508	*+0·604*	*−0·625*	+0·107
12 ft. . .	−0·375	+0·373	*−0·652*	+0·093
Sum 3 levels .	−0·465	*+0·643*	*−0·681*	+0·043

Correlations with a significance greater than $P = 0{\cdot}05$ are in italics.

bark up to a height of 12 ft.). This positive correlation indicates that a smaller crop is associated with a smaller content of potassium in the bark, and contrasts with the relationship found between sucrose and crop, suggesting that *potassium is the factor most likely limiting crop size.* In the wood the seasons correlation between crop and potassium is negative at all levels and significant at 6 ft. and 12 ft. and sum of levels, indicating that the potassium reserves of the wood as well as those of the bark are drawn upon by the developing fruits. The residual correlations of wood potassium and crop are low and not significant.

In connexion with the potassium reserves of the cacao tree it may be recalled that, during an investigation into the mineral content of leaves on trees close to the present experimental trees (Humphries, 1940), the leaves of a new flush drew more potassium (44 per cent. of the amount originally present) from the leaves of the next older flush than any other element, an observation which supports the contention that potassium tends to be limiting on the site of these experiments.

In order to see if the picture is substantially altered when the mutual effects of several of the variables are eliminated, the fourth-order correlation coefficients have been calculated. The six variables concerned are sucrose, starch, reducing sugars, potassium, phosphorus, and crop. The first five are expressed as a concentration (g. per 100 g. water) and the latter as g. dry weight. In extracting the partial correlations between sucrose and crop and potassium and crop, interesting relationships between certain other components were revealed and these will be briefly discussed.

The fourth-order seasons correlations are shown in Table IV. The correlation between potassium of the bark and crop is negative at all levels but not significant at any level, just approaching significance at 6 ft. There is a significant negative correlation between potassium of the wood (sum of levels) and crop. The seasons correlation between sucrose and crop is also negative

TABLE IV

Fourth-order Seasons Correlations between Sucrose, Starch, Total Reducing Sugars, Potassium, Phosphorus, and Crop in the Bark and Wood at Three Levels

	Bark.				Wood.			
	1 ft.	6 ft.	12 ft.	Sum.	1 ft.	6 ft.	12 ft.	Sum.
Sucrose × Starch	*−0·720*	*+0·671*	—	*+0·705*	—	—	—	—
Sucrose × K	—	—	—	*−0·618*	—	—	—	—
Sucrose × P	*+0·609*	—	—	—	+0·563	—	—	—
Starch × T.R.S.	*+0·621*	—	*−0·765*	—	—	—	—	—
Starch × K	—	*+0·674*	*+0·733*	*+0·726*	+0·576	—	—	*+0·607*
Starch × P	*+0·809*	—	*+0·738*	*+0·671*	—	—	—	—
T.R.S. × K	—	—	—	—	—	—	—	−0·586
T.R.S. × P	*−0·724*	—	*+0·769*	—	—	—	—	—
Sucrose × Crop	*−0·694*	*−0·632*	—	−0·549	—	—	—	—
K × Crop	—	−0·596	—	−0·538	−0·566	−0·581	—	*−0·700*
Starch × Crop	−0·571	—	—	—	—	—	—	—

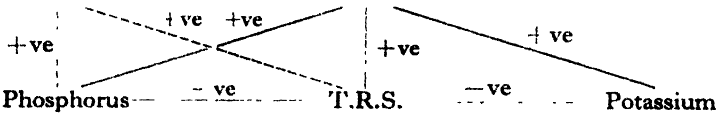
Only correlations with significance greater than $P = 0·1$ are shown. Correlations with significance greater than $P = 0·05$ are in italics.

and significant at the 1-ft. and the 6-ft. levels of the bark. The correlation between starch and crop is not significant at any level. This may be due to the fact that starch in some regions of the bark is not mobilized so that the 'static' starch has a large masking effect. The residual correlation coefficients (Table V) between potassium of the bark and crop are positive at all levels and are significant for the 1-ft. level and for the three levels together. In the wood, however, the correlations are not significant. The residual correlations between sucrose and crop, on the other hand, are negative both in bark and wood and significant at 1 ft., 12 ft., and sum of levels in the bark and at 1 ft. in the wood. Thus the conclusion that potassium limits crop production, while carbohydrate does not, is substantiated.

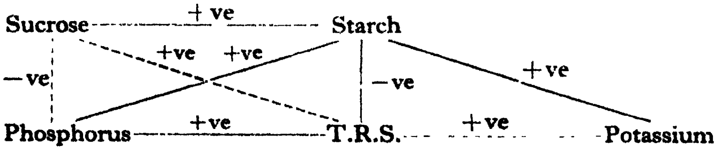
Certain of the other fourth-order seasons correlations shown in Table IV are of interest. Sucrose and starch of the bark show a significant negative correlation at the 1-ft. level but a significant positive correlation at 6 ft. The correlation in the case of the composite sample (sum of levels) is positive and significant. This diametrically opposite relationship between these carbohydrates at the two levels is matched by the case of starch and total reducing sugars. At the 1-ft. level of the bark the correlation is positive and significant while at 12 ft. it is negative and highly significant. The correlation between total reducing sugars and total phosphorus of the bark is negative and significant at 1 ft. and positive and significant at 12 ft. The relationship between starch and phosphorus, on the other hand, is consistently positive and significant at both 1 ft. and 12 ft. and at sum of levels. This is also the case between starch and potassium (significant at 6 ft., 12 ft., and sum of levels in the bark and also at sum of levels in the wood). There are very few significant correlations between components in the wood, but in general they show the same trend as in the bark, although there is no clear evidence of a change from positive to negative values or vice versa at different levels.

The seasons correlations between components of the bark reserves are summarized in the following diagram, where significant correlations between two components are indicated by continuous lines and non-significant correlations by broken lines:

1-ft. level



Higher levels



Although not significant, the change of sign of the correlations is also evident in the cases of Sucrose × Phosphorus and Total Reducing Sugars × Potassium.

The reason for the difference in behaviour at the two levels is not altogether evident and a full explanation is probably not possible with the present data. It may be suggested, however, that the 1-ft. level behaves differently from regions higher in the tree because it is contiguous to the root and possibly, like the root, is predominantly a storage region and hence essentially a region of synthesis.

The positive correlation between starch and potassium is evident both in the bark and the wood. A relationship between starch and potassium has been found in numerous other investigations, but so far no adequate theory of the mechanism of their association is forthcoming. No doubt the positive correlation between starch and phosphorus is connected with the phosphorylation reactions.

Considering now the remainder of the fourth-order residual correlations (Table V), sucrose and starch are negatively correlated in the bark at the 1-ft. level, a general inverse correlation between these two carbohydrates which is to be expected. Sucrose and potassium are positively correlated at the 1-ft. level in the bark, i.e. trees with a high potassium content would tend to have a high sucrose content. On the other hand, trees with a high phosphorus content would tend to have a low sucrose content at the 1-ft. level in the bark. Potassium and phosphorus are positively correlated at the 1-ft. level of the bark.

TABLE V

Fourth-order Residual Correlations between Sucrose, Starch, Total Reducing Sugars, Potassium, Phosphorus, and Crop in the Bark and Wood at Three Levels

	Bark.				Wood.			
	1 ft.	6 ft.	12 ft.	Sum.	1 ft.	6 ft.	12 ft.	Sum.
Sucrose × Starch	*−0·579*	—	—	—	—	—	—	—
Sucrose × K	*+0·648*	—	—	—	—	—	—	*−0·661*
Sucrose × P	*−0·674*	—	—	—	—	—	—	—
Starch × K	—	—	—	—	*−0·577*	—	—	—
Starch × P	—	—	+0·529	—	—	—	—	—
T.R.S. × P	—	*+0·644*	—	*+0·745*	—	—	—	—
K × P	*+0·606*	—	+0·563	+0·525	—	—	—	—
Sucrose × Crop	*−0·879*	—	*−0·630*	*−0·674*	*−0·575*	—	—	−0·545
K × Crop	*+0·761*	+0·502	—	*+0·647*	—	—	—	—
Starch × Crop	−0·553	—	—	—	—	—	—	—
P × Crop	*−0·660*	—	—	—	—	—	—	—

Only correlations with significance greater than $P = 0{\cdot}1$ are shown. Correlations with significance greater than $P = 0{\cdot}05$ are in italics.

It is convenient at this point to sum up the main conclusions from this series of papers concerned with the physiological wilt of cacao fruits. It has been shown that wilt is due to competition between fruits for mineral nutrients. No evidence was found that carbohydrate shortage was a cause of wilt.

Competition was illustrated by the fact that fruits formed early in the season had the greatest chance of survival and of reaching maturity. Fruits formed successively as the season advanced wilted at an increasingly younger age. Fruits formed on thinner and more distant branches had less chance of survival than those formed on the thicker and older parts, i.e. those parts with the greatest reserves of nutrients. It was also shown that competition for nutrients by the foliage may be an important factor in determining survival in young trees. It is concluded that potassium (the element present in greatest amount in the cacao fruit) is chiefly, if not entirely, responsible for diminished crop in the trees investigated.

SUMMARY

The seasonal changes in potassium, phosphorus, nitrogen, and calcium in the bark and wood of the cacao tree at levels of 1 ft., 6 ft., and 12 ft. from the base of the trunk have been followed over a period of 14 months.

There was a significant seasonal variation in potassium and phosphorus of the bark and wood. The seasonal variation in total nitrogen and calcium was significant in the bark but not in the wood.

A negative gradient of potassium was present in the wood and the seasonal changes were correlated with changes in the bark.

There was a negative gradient of calcium in the wood which decreased to a marked extent during the latter part of the dry season. This is held to be evidence that uptake of soil solutes practically ceased at the height of the dry season.

The concentration of potassium in the bark was highest at the 12-ft. level and lowest at the 6-ft. level, so that there was a negative gradient of potassium between 1 ft. and 6 ft. and a positive gradient between 6 ft. and 12 ft. It is assumed that the former is predominantly a structural and storage gradient while the latter is a dynamic transport gradient. The dynamic gradient increased and the storage gradient decreased at times of greatest demands on the potassium reserves. A negative gradient of total nitrogen between 1 ft. and 6 ft. and a positive gradient between 6 ft. and 12 ft. was also found. A similar state of affairs existed in the case of the non-carbohydrate alcohol-soluble material. This fraction, consisting in part of soluble nitrogenous material, will to a certain extent be concerned with movement in the bark. As in the case of potassium, the largest positive gradients occur at periods of active utilization of reserves.

An analysis of covariance between mineral constituents of the bark or wood and crop (expressed as g. dry weight) showed that potassium was depleted by seasonal increase in crop whereas the correlation between potassium and crop at an instant was positive—evidence that potassium limited crop size. Fourth-order correlation coefficients between the variables showed substantially the same picture and also indicated that certain pairs of components were correlated in an opposite manner at different levels.

I am indebted to Dr. D. J. Watson for critically reading the manuscript.

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APPENDIX, TABLE I

Seasonal Trend in Potassium Content (g. per 100 g. Water) of Bark and Wood at Three Levels

	Bark.						Wood.					
	1 ft.		6 ft.		12 ft.		1 ft.		6 ft.		12 ft.	
Sampling date.	A	B	A	B	A	B	A	B	A	B	A	B
Nov. 10	0·29	0·31	0·25	0·32	0·28	0·41	0·48	0·48	0·28	0·42	0·23	0·40
Dec. 16	0·42	0·47	0·39	0·44	0·49	0·39	0·42	0·53	0·30	0·39	0·35	0·35
Jan. 12	0·36	0·55	0·44	0·47	0·50	0·53	0·59	0·50	0·46	0·45	0·41	0·37
Feb. 9	0·48	0·53	0·46	0·48	0·52	0·49	0·53	0·57	0·44	0·57	0·46	0·49
Mar. 9	0·43	0·57	0·43	0·53	0·52	0·54	0·54	0·57	0·47	0·48	0·36	0·41
Apr. 6	0·69	0·46	0·41	0·48	0·46	0·54	0·61	0·54	0·38	0·52	0·41	0·42
May 5	0·53	0·43	0·53	0·35	0·56	0·42	0·50	0·46	0·47	0·64	0·54	0·35
June 3	0·44	0·41	0·46	0·35	0·43	0·44	0·52	0·41	0·48	0·65	0·40	0·54
June 29	0·44	0·50	0·37	0·36	0·42	0·48	0·46	0·58	0·45	0·42	0·41	0·42
July 27	0·45	0·40	0·49	0·34	0·49	0·41	0·57	0·61	0·53	0·47	0·42	0·40
Aug. 24	0·42	0·50	0·49	0·46	0·53	0·48	0·57	0·51	0·62	0·49	0·50	0·39
Sept. 28	0·44	0·39	0·50	0·36	0·50	0·45	0·52	0·58	0·47	0·43	0·43	0·40
Oct. 26	0·44	0·36	0·40	0·28	0·48	0·38	0·50	0·44	0·35	0·36	0·30	0·33
Dec. 1	0·45	0·27	0·38	0·27	0·38	0·35	0·43	0·41	0·37	0·25	0·33	0·24

APPENDIX, TABLE II

Seasonal Trend in Calcium Content (g. per 100 g. Water) of Bark and Wood at Three Levels

	Bark.						Wood.					
	1 ft.		6 ft.		12 ft.		1 ft.		6 ft.		12 ft.	
Sampling date.	A	B	A	B	A	B	A	B	A	B	A	B
Nov. 10	4·60	3·98	3·93	3·92	3·41	3·08	1·11	0·90	1·01	0·80	0·89	1·24
Dec. 16	3·92	3·43	2·95	3·34	1·96	3·51	0·84	0·90	1·01	0·70	0·92	0·78
Jan. 12	4·47	3·15	4·05	3·08	3·15	2·82	0·97	0·87	1·10	0·71	0·81	0·67
Feb. 9	4·21	3·97	3·85	3·39	2·88	3·26	1·29	0·84	0·94	0·82	0·75	0·73
Mar. 9	4·39	4·79	4·03	3·89	3·51	3·38	1·16	1·10	1·38	1·10	1·06	1·03
Apr. 6	3·91	3·96	3·48	3·98	2·91	2·59	0·88	1·09	0·73	0·84	0·82	0·95
May 5	3·65	4·25	3·56	4·07	2·78	3·57	0·83	0·78	1·07	1·10	0·81	0·81
June 3	3·58	4·34	3·31	4·19	3·07	3·30	0·90	0·85	0·77	1·37	0·70	1·19
June 29	3·88	4·54	4·04	4·28	3·52	3·17	0·59	1·19	0·71	1·14	0·56	0·71
July 27	4·31	4·51	3·66	4·33	3·91	3·34	1·01	1·10	1·09	0·89	0·71	0·78
Aug. 24	4·05	3·94	3·25	3·27	3·24	3·47	0·66	1·12	0·90	0·98	0·56	0·83
Sept. 28	4·29	4·28	4·19	3·71	2·82	3·41	1·11	1·03	1·08	0·80	0·79	0·65
Oct. 26	4·21	4·28	3·80	4·19	3·46	3·38	1·06	0·87	0·84	0·85	0·77	0·58
Dec. 1	3·74	4·52	3·83	4·12	2·44	3·61	0·92	0·83	0·61	0·69	0·68	0·60

APPENDIX, TABLE III

Seasonal Trend in Total Nitrogen Content (g. per 100 g. Water) of Bark and Wood at Three Levels

	Bark.						Wood.					
	1 ft.		6 ft.		12 ft.		1 ft.		6 ft.		12 ft.	
Sampling date.	A	B	A	B	A	B	A	B	A	B	A	B
Nov. 10	0·46	0·45	0·43	0·42	0·45	0·41	0·40	0·31	0·37	0·35	0·42	0·43
Dec. 16	0·35	0·40	0·35	0·34	0·32	0·37	0·31	0·40	0·30	0·33	0·30	0·31
Jan. 12	0·44	0·38	0·37	0·34	0·38	0·35	0·36	0·31	0·28	0·31	0·32	0·34
Feb. 9	0·41	0·40	0·37	0·36	0·34	0·37	0·44	0·39	0·47	0·31	0·55	0·47
Mar. 9	0·43	0·44	0·40	0·36	0·37	0·35	0·35	0·37	0·43	0·38	0·41	0·45
Apr. 6	0·43	0·40	0·41	0·36	0·43	0·38	0·47	0·35	0·52	0·35	0·52	0·34
May 5	0·39	0·37	0·32	0·38	0·40	0·31	0·28	0·36	0·29	0·57	0·32	0·44
June 3	0·36	0·41	0·33	0·36	0·37	0·51	0·35	0·35	0·29	0·58	0·34	0·34
June 29	0·37	0·41	0·38	0·43	0·39	0·38	0·34	0·39	0·29	0·42	0·33	0·46
July 27	0·39	0·39	0·34	0·37	0·39	0·36	0·37	0·47	0·35	0·35	0·29	0·42
Aug. 24	0·35	0·43	0·27	0·37	0·40	0·41	0·25	0·36	0·29	0·35	0·30	0·41
Sept. 28	0·38	0·40	0·39	0·38	0·40	0·41	0·37	0·36	0·38	0·35	0·37	0·51
Oct. 26	0·37	0·36	0·33	0·37	0·36	0·37	0·25	0·35	0·35	0·33	0·34	0·35
Dec. 1	0·37	0·41	0·40	0·37	0·34	0·39	0·39	0·35	0·42	0·38	0·36	0·37

APPENDIX, TABLE IV

Seasonal Trend in Phosphorus Content (g. per 100 g. Water) of Bark and Wood at Three Levels

	Bark.						Wood					
	1 ft.		6 ft.		12 ft.		1 ft.		6 ft.		12 ft.	
Sampling date.	A	B	A	B	A	B	A	B	A	B	A	B
Nov. 10	0·028	0·028	0·025	0·036	0·029	0·035	0·058	0·048	0·044	0·050	0·043	0·051
Dec. 16	0·030	0·026	0·025	0·037	0·027	0·028	0·044	0·053	0·037	0·038	0·041	0·040
Jan. 12	0·029	0·027	0·029	0·029	0·028	0·035	0·059	0·045	0·028	0·034	0·027	0·038
Feb. 9	0·038	0·024	0·040	0·032	0·040	0·037	0·054	0·050	0·047	0·042	0·049	0·048
Mar. 9	0·029	0·032	0·032	0·032	0·031	0·032	0·044	0·046	0·035	0·044	0·036	0·040
Apr. 6	0·029	0·027	0·032	0·026	0·026	0·026	0·049	0·058	0·041	0·043	0·030	0·033
May 5	0·033	0·032	0·034	0·031	0·032	0·029	0·043	0·059	0·048	0·054	0·043	0·043
June 3	0·028	0·033	0·029	0·034	0·030	0·034	0·052	0·071	0·041	0·054	0·045	0·055
June 29	0·029	0·032	0·030	0·034	0·034	0·033	0·057	0·067	0·036	0·067	0·035	0·057
July 27	0·032	0·031	0·033	0·036	0·033	0·029	0·062	0·059	0·054	0·054	0·051	0·080
Aug. 24	0·027	0·031	0·023	0·029	0·025	0·028	0·043	0·055	0·037	0·049	0·034	0·055
Sept. 28	0·031	0·031	0·030	0·030	0·031	0·034	0·065	0·060	0·050	0·055	0·045	0·053
Oct. 26	0·031	0·031	0·030	0·028	0·030	0·031	0·058	0·047	0·044	0·039	0·035	0·035
Dec. 1	0·029	0·027	0·033	0·028	0·030	0·031	0·055	0·048	0·042	0·038	0·044	0·037

Occurrence of Hermaphrodite Flowers and the Development of Female Gametophyte in *Ephedra intermedia* Shrenk et Mey.

BY

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With Plates V and VI and seventeen Figures in the Text

INTRODUCTION

EPHEDRA INTERMEDIA occurs in a state of nature in the north-west Himalayas, Kashmir, and western Tibet at an altitude of 8,000–14,000 ft. above sea-level. Stapf (1889) describes its distribution also from north-east Baluchistan, Afghanistan, and Pamir. It grows in exposed and arid conditions in calcareous soil and among stone crevices in rather inaccessible places. The plant attains a height of about 2 ft. The branches are erect or ascending and the ridges and grooves on them are feebly developed. In Lahul the flowering period was observed by the writer to begin in early June. Pollination occurs in the first week of July and the seeds ripen by the end of September.

The material for the present study was almost exclusively obtained from plants raised from seed in the Government College Botanic Garden at Lahore, Panjab. The seeds were obtained from Chini Range in Bashahr Division through the courtesy of Mr. I. P. Mohan of the Forest Department, to whom the writer wishes to express his thanks. The species has shown a remarkably good growth and has attained a height of more than 3 ft. The plants began to bear flowers after 3–4 years. There has been an alteration in growth and flowering period from that in its natural home in adaptation to the climatic conditions of the Panjab. Here the species begins to sprout out fresh shoots about the last week of February with the arrival of spring. The flowers appear in the middle of March, pollen is shed about mid-April, while the seeds ripen in the last week of June. The other exotic species of the genus that have been cultivated at Lahore, *E. altissima* Desf., *E. saxatilis* Royle, *E. sinica* Stapf, *E. likiagenis* Florin, likewise show a similar growth and flowering period, although the exact time of flowering, ripening of pollen, or maturity of seeds varies somewhat in the different species. This flowering and growth period coincides with that observed for the native species of the genus *E. foliata* Boiss.

This species is of some economic importance. It ranks next to *E. sinica*, *E. gerardiana*, *E. nebrodensis*, and *E. equisetina* as a source of the valuable drug ephedrine. Read and Feng (Chopra, 1933) have estimated the ephedrine content of the species to be nearly 0·4 per cent., while Chopra finds it to be

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in the neighbourhood of 0·2 per cent. The proportion of pseudo-ephedrine, however, is greater than that of ephedrine.

The fixation was mostly done in formaline-acetic-alcohol. This fixative yielded better results than either Bouin's or Nawaschin's as it caused little plasmolysis at the free nucleate embryo-sac stage. Sections were cut from 10 to 20μ thick, depending on different stages. The staining was done with Safranin 'o' in combination with either Fast Green or Gentian Violet.

CONE STRUCTURE

The plants that have developed from seeds are polymorphic. Some bear only male cones, others bear only female cones, while still others are monoecious and bear male, female, and bisexual cones.

Male cone

As in *E. saxatilis*, 2, 3, or 4 cones may be borne at a node. Each cone possesses a stalk up to an inch long. In *E. intermedia* most of the vegetative shoots possess 2 leaves at each node, but there are others more strongly developed which possess 3 leaves at each node. The cones borne on the former type of branch invariably possess a pair of bracts at each node, or in other words they are 'bimerous'. There are 4–5 such pairs decussately arranged, of which the lowest one or two are sterile while the rest are fertile. On the other hand, cones borne on the stouter branches always have bracts borne in 3's at each node, i.e. they are 'trimerous'. There are 4 or 5 such tiers of bracts, of which the lowest one or two are sterile. The bracts of successive tiers alternate.

As usual in the genus, each male flower possesses two perianth leaves arranged anterio-posteriorly enclosing within a central column-like structure or the sporangiophore. The latter bears at the apex 6–8 biolocular sporangia, each of which is shortly stalked. In many cases the sporangiophore itself is bifurcate near the apex and in that case each shank bears 3–4 stalked bilocular sporangia.

Female cone

The female cones are terminal on freshly formed young branches. These branches arise in a whorl of 3–4 at a node in the axils of leaves of previous year's shoots. Like the male cones the female cones may be 'bimerous' or 'trimerous' according as they arise on shoots with 2 leaves at a node or 3 leaves at a node. Each cone bears 3–4 whorls of bracts. Usually, all excepting the uppermost pair of bracts are sterile. The 'dimerous' cone has two ovules, one in the axil of each fertile bract. Often one of these two is abortive. In a 'trimerous' cone there are commonly three ovules, one in the axil of each bract. Similar 'bimerous', 'trimerous', and occasionally 'tetramerous' cones have been observed in *E. foliata* and *E. altissima* growing in the Botanic Garden at Lahore borne on shoots with 2, 3, or 4 leaves at each node respectively.

The two ovules borne on 'bimerous' cone are as a rule free from one another. Not uncommonly the outer integument of the two ovules may be partially or completely fused on the flat side facing each other. If the fusion is complete it gives the appearance of a single large ovule, but the inner integuments of the two ovules are actually free and their respective micropylar tubes are distinct. It appears that this fusion is the result of a common basal growth at the two closely situated growing regions of the outer integument in the ovule primordia. Similar fusion of the outer integuments has also been observed in 'trimerous' cones involving all the three ovules or only two of them.

Such partial or complete union of the outer integuments is not limited only to the present species. It has been observed in *E. saxatilis* and *E. foliata.* It reminds one of a similar condition occurring in the ovules of *Ginkgo biloba* resulting in the formation of 'double ovules' (Mehra, 1938).

While as a rule only the uppermost whorl of bracts is fertile, in rare cases ovules may be borne in the axils of the bracts of the next lower node (Text-fig. 1). Similar features have also been observed in *E. saxatilis* and *E. sinica.* In the latter species both the bracts of the next lower node may bear an ovule each.

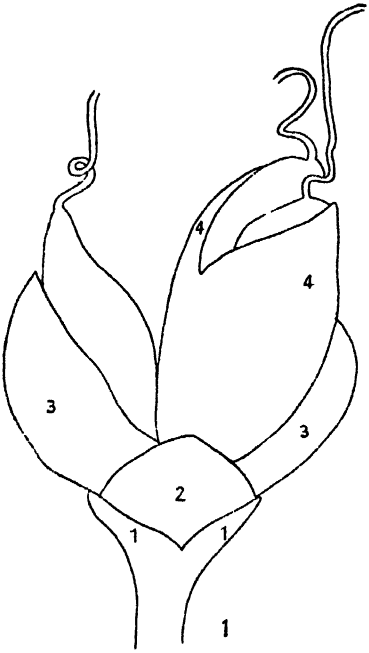
Bisexual cones

Monoecious plants bear male, female, and bisexual cones. There is no regularity in their distribution on the branches. Often two or all the three different types of cones may be borne in a group at the apex of the same branch. In such cases the terminal cone is usually female and those below possess different sexuality. A terminal female and a lateral male is illustrated in Pl. V, Fig. 1 *b*, while a terminal female and a lateral bisexual are shown at *c*. A terminal female, one lateral male, and another lateral bisexual are shown in Pl. V, Fig. 1 *d*. In other cases there may be a terminal female with both laterals of male sex.

In bisexual cones the ovules are borne in the axil of the uppermost pair of bracts; the next pair is usually sterile followed by a pair of bracts bearing male flowers and ending in a pair of sterile bracts (Text-fig. 2). The same is observed in Pl. V, Fig. 1 *a*, but in this bisexual cone only one of the second pair of bracts bears a male flower while the corresponding other is sterile. A 'trimerous' bisexual cone is shown in Text-fig. 3 with five verticils of bracts. The two lowest are sterile followed by a whorl bearing male flowers. This is separated from the uppermost ovule-bearing whorl by a whorl of sterile bracts.

This is not the first record of the occurrence of bisexual cones in the species. A passing reference to this effect is made by Pearson (1929). Bisexual cones are also known in three other species of the genus, namely *E. campylopoda*, *E. americana* var. *Humboldtii*, and *E. trifurca.*

Recently bisexual cones have been observed by the writer in *E. sinica* from plants raised from seeds, although this feature has not so far been recorded.



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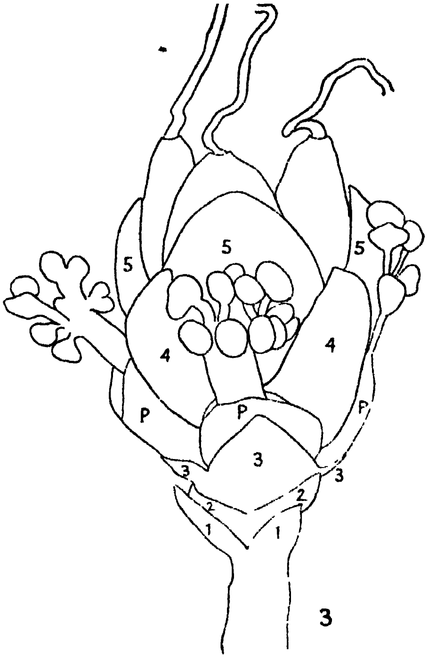

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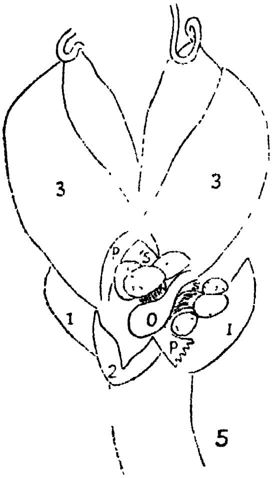

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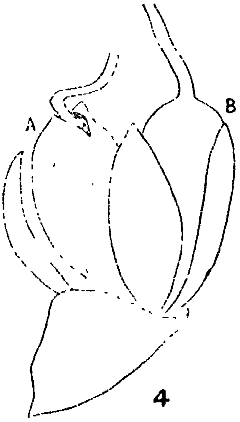

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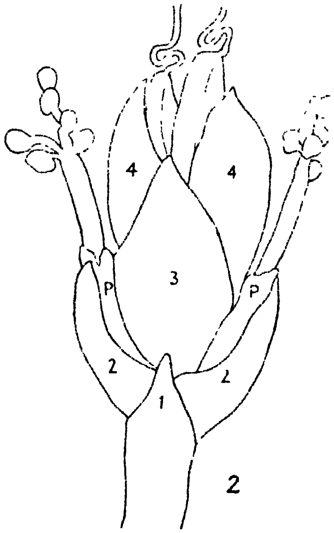

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FIG. 1. A female cone with the uppermost pair of bracts marked '4' fertile, each bearing an ovule in its axil. One bract of the next lower pair is also fertile.

FIG. 2. A 'bimerous' bisexual cone, with shortly stalked sporangia on the male flowers.

FIG. 3. A 'trimerous' bisexual cone.

FIG. 4. Two ovules that were borne at the apex of a bisexual cone, the one on the left marked 'A' with distinctly two lipped outer integument. The one on the right marked 'B' had the outer integument cleft to the base on the side facing the reader, on the other side it was bifid.

FIG. 5. A bisexual cone bearing a *hermaphrodite flower*. Of the three decussately arranged pairs of bracts, the lowest marked '1' sterile. Uppermost pair marked '3' each bearing an ovule. Of the middle pair, the one facing the reader marked '2' fertile, while the corresponding on the other side is sterile. In the axil of the former a *hermaphrodite flower* showing two median perianth leaves P, two stamens S, 'S enclosing in the centre an ovule O.

Certain interesting ovules

These deserve special mention. They are somewhat weakly developed and show a striking external resemblance to young male flowers. They are borne either in the axils of uppermost bracts of some bisexual cones or in the axils of the bracts next to the uppermost in some female cones.

It is a well-known fact that a normal ovule in *Ephedra* possesses two integuments, an outer thick and fleshy one completely surrounding the ovule like a collar, and an inner thin and papery one, produced above into a long micropylar tube which is tortuous in *E. intermedia*. Both the integuments are free from one another. The morphology of the outer integument has been in dispute.

The ovules in question show certain features that throw some light on this problem. Two of these borne at the apex of a bisexual cone are drawn on a magnified scale in Text-fig. 4. The outer integument of the ovule on the left marked 'A' is whitish-hyaline, more or less membranous, and distinctly two-lipped at the apex. It appears as if it is formed of two membranous leaf-like structures which are united all along the margins excepting near the apex. Each of these leaf-like structures bears a feebly developed mid-rib. These 'leaves' arise laterally. In the other ovule on the right, marked 'B', the outer integument is completely cleft as far as the base of the ovule on one side (the front side in the diagram), while on the other side it is bifid. The micropylar tube in these ovules is ill developed and is straight or weakly tortuous but the ovule is normal inside.

These ovules irresistibly suggest a homology between the outer integument of the ovule and the perianth leaves of the male flowers. They strongly suggest that the outer integument is in reality of the nature of two fused perianth leaves which have formed a collar-like covering around the ovule proper. These 'perianth leaves', however, arise laterally in contrast to the anterio-posterior arrangement of the perianth in the male flower.

That the outer integument is not really a part of the ovule proper is further shown by the fact that in the abnormal hermaphrodite flowers recorded below for the species, the ovule present in the centre possesses only a single integument—the so-called inner integument.

HERMAPHRODITE FLOWERS

A striking feature in the species is the occasional occurrence of hermaphrodite flowers. Hermaphrodite flowers have not so far been reported in any species of the genus. The rule is the formation of strictly male or strictly female flowers. The hermaphrodite flowers in this species are borne on bisexual cones. Two of these cases may be illustrated.

In Text-fig. 5 is drawn a cone with three pairs of bracts. The lowermost pair marked '1' is sterile, while the uppermost marked '3' bears two ovules, one in the axil of each bract. Of the middle pair, one of the bracts not seen in the figure (being on the opposite side) is sterile while the other bears a *hermaphrodite flower.* The flower was not fully mature and externally appeared like a young unopened male flower covered over by two anterioposteriorly arranged perianth leaves marked 'P'. On dissection it showed two stalks, one marked 'S' bearing three bilocular sporangia (all of which are not shown) and the other ''S' with two sporangia, one of which is bilocular and the other trilocular. In the centre of the flower is a rudimentary ovule with its only integument produced above into a moderately long micropylar tube.

Another case is illustrated in Pl. V, Fig. 2, from a sectioned cone. In the centre of the flower is observed a poorly developed ovule with its integument produced into a long micropylar tube. On the outside are two stamen stalks bearing in all six bilocular sporangia. One of these stalks is clear in the photograph while the other is in the adjacent section. The whole thing is enveloped on the outside by two perianth leaves. The nucellus of the ovule is yet undifferentiated, while the sporangia are well developed and in these the microspore mother cells are about to be formed.

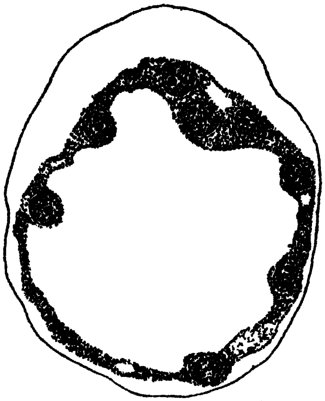
The theoretical significance ascribed to the occurrence of these highly interesting hermaphrodite flowers is dealt with later in discussion.

DEVELOPMENT OF THE FEMALE GAMETOPHYTE

The archesporial cell is definitely hypodermal in origin. In *E. saxatilis* the appearance of two hypodermal archesporial cells is not infrequent (Mehra, unpublished). The overlying epidermal cell divides at first by a periclinal wall (Text-fig. 6). This is followed by more periclinal and anticlinal wall forming a multicellular tissue that submerges the archesporial cell deep inside the nucellus. The archesporial cell never cuts off a parietal cell, as has been reported by Maheshwari (1935) in *E. foliata.* It becomes directly transformed into a megaspore mother-cell. The overlying epidermal tissue is thus entirely the product of division of the epidermal cell and not the joint product of division of the epidermal cell and the parietal cell, as maintained by Maheshwari for *E. foliata.*

The megaspore mother-cell undergoes the first reduction division in the usual manner. The leptotene stage has threads chromomeric in nature and the diplotene stage is drawn in Text-fig. 7. The cytoplasm around the enlarged nucleus in the latter stage is fibrillar in appearance. Both the diads undergo a simultaneous division (Text-fig. 8) forming a linear tetrad of four megaspores. Not infrequently the upper diad lags behind and may even degenerate without dividing, while the lower diad invariably forms a row of two megaspores (Text-fig. 9).

The lowest of these megaspores is functional. The earlier stages in the development of the female gametophyte are those characteristic of gymnosperms in general excepting *Welwitschia.* The megaspore enlarges, its nucleus divides into two. A vacuole soon appears in the centre pushing the two nuclei towards the two poles. As the embryo-sac enlarges further, the peripheral



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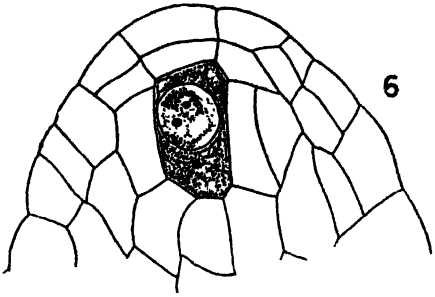

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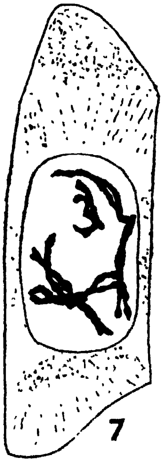

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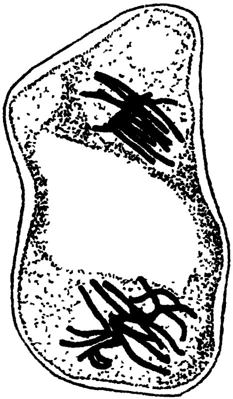

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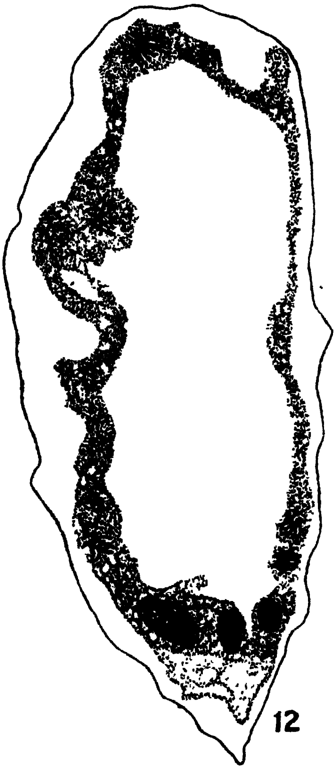

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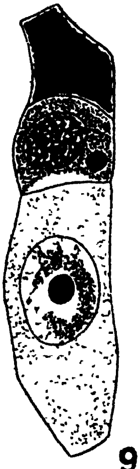

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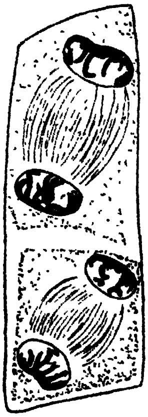

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FIG. 6. Hypodermal archesporial cell. The overlying epidermal cell has divided by a periclinal wall. (×619.)

FIG. 7. Mother cell at diplotene. (× 1,310.)

FIG. 8. Both diads dividing simultaneously. (× 1,310.)

FIG. 9. Upper diad degenerated. Of the two spores of the lower diad, upper one in the process of degeneration while lower functional. (× 1,310.)

FIG. 10. Simultaneous division in the 2-nucleate embryo-sac. (× 1,310.)

FIG. 11. A section through a 32-nucleate embryo-sac. (× 350.)

FIG. 12. Polarity in the division of the nuclei from 64 to 128-nucleate embryo-sac. Those at the chalazal end still at prophase while the micropylar ones at metaphase. (× 220.)

nucellar cells are crushed and partly digested, appearing in sections as darkly stained degenerate masses. The passage from 2- to 4-nucleate stage is seen in Text-fig. 10, both the nuclei being at the metaphase stage. Text-fig. 11 depicts the 32-nucleate stage. The embryo-sac now undergoes a considerable distension in size and then its nuclei divide simultaneously to enter into a 64-nucleate stage. Up to this stage the divisions of the nuclei in the embryo-sac are simultaneous. From now onwards strict regularity is not maintained. In many cases a distinct polarity is visible, the nuclei towards the micropylar end being at a more advanced stage of division than those towards the chalazal end. In an embryo-sac entering into a 128-nucleate stage from 64-nucleate stage, the nuclei towards the micropylar end have entered the metaphase, while the chalazal ones are yet in early prophase (Text-fig. 12).

In the later free-nucleate stages one or more nuclei may become inert and no longer participate in further divisions. These degenerate with obvious repercussions on the total number of nuclei in the succeeding stages, which no longer remain strict theoretical multiples of two.

Wall formation is initiated, as a rule, at 'the 256-nucleate' stage, i.e. after 8 generations of nuclear divisions from the megaspore, when from 210 to 240 nuclei can be counted. But there is considerable variability in this feature. Sometimes walls are observed to be initiated in embryo-sacs in which more than 450 nuclei can be counted, which means after 9 generations of nuclear divisions from the megaspore (Text-fig. 15). This fluctuation in the stage of wall formation in the species is interesting.

A study of literature reveals that the process of wall formation has not been studied in any species of the genus so far. In *Ephedra intermedia* at the close of the free nuclear period a membrane arises on the outer border of the peripheral layer of cytoplasm in which the free nuclei are embedded. This cytoplasm now assumes a fibrillar appearance. Around each nucleus radiating masses of fibres make their appearance as seen from the tangential view. This is followed by the accumulation of cytoplasm in the central region between the adjacent nuclei where the fibrils belonging to the one meet those of the others. In these regions the walls are laid down. Thus polygonal cells are organized as seen in the tangential view. The radial walls of these cells abut directly on the peripheral membrane of the embryo-sac. These cells are at first open towards the interior of the embryo-sac (Text-fig. 15) and at these regions there is dense cytoplasm. When wall formation is complete the embryo-sac is filled by elongated or 'alveolar' cells (Text-fig. 16). Each alveolar developing centripetally cell contains a single nucleus, whose position varies.

When nuclear division has been simultaneous at the last free-nucleate stage, the beginning of wall formation around each nucleus is also simultaneous. In cases where there has been a polarity in division at the last free-nucleate stage, this is continued in the time of wall formation around the nuclei at the two ends of the sac. Thus in Text-fig. 14 the nuclei towards the micropylar end have become enclosed by walls, while no such indication is visible towards the lower end.

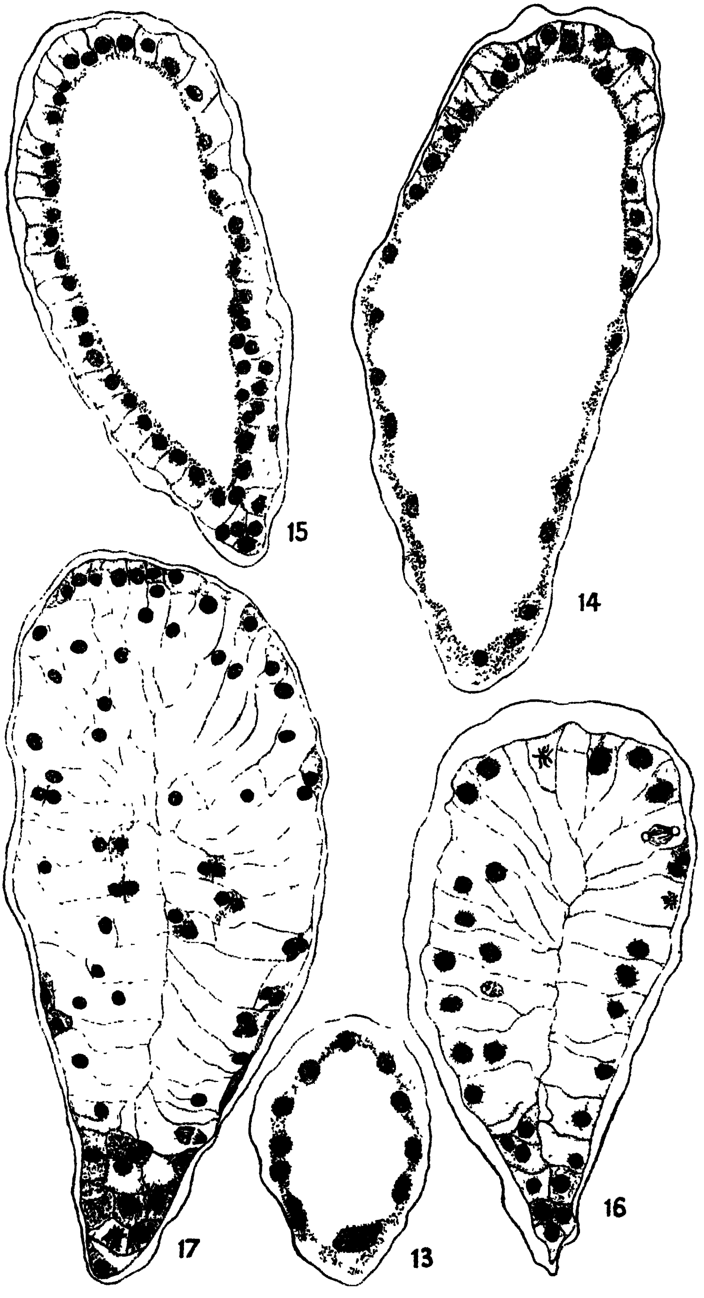

FIG. 13. A section through a '64-nucleate' embryo-sac (in which 57 nuclei were counted) showing a very large nucleus at the chalazal end probably formed by the fusion of two nuclei. (× 171.)

FIG. 14. Polarity in the laying down of walls in an embryo-sac in which a total of 204 nuclei was counted. (× 171.)

FIG. 15. Wall formation at '512-nucleate' stage (more than 450 nuclei were counted). (× 171.)

FIG. 16. Alveolar method of wall formation. (× 171.)

FIG. 17. Transverse segmentation of alveoli. (× 171.)

After the gametophyte has become completely alveolar the transverse division of the alveoli begins. The place at which the partition is laid down in an alveolus is determined by the position of its nucleus when division occurs. This is variable. In any case the spindle of the mitotic figure is very small and the two daughter nuclei are observed touching the newly formed wall on either side. Further divisions ensue, and every time a wall is laid down so that each cell organized is uninucleate. This further division of the gametophyte is much more active towards the lower end than towards the micropylar end.

The young gametophyte at this stage is conical in shape, broad above and narrow below. It can be differentiated approximately into two halves. The upper half is composed of radially elongated, large, thin-walled hyaline cells with scanty cytoplasm. This gradually merges into the tissue of the lower half of the gametophyte composed of compact, more or less polygonal cells of smaller size with dense contents. The multiplication of cells in both the regions of the gametophyte continues, more so in the lower region than in the upper, with the simultaneous increase in size of the gametophyte as a whole. By the time the archegonia are mature, the lower half of the gametophyte has again undergone a further differentiation into an upper region of polygonal cells undergoing a rather active division, and a basal zone composed of relatively larger cells packed with dense cytoplasm and containing rather large nuclei. The latter cells are apparently haustorial in function, drawing nourishment from nucellar cells below and passing it on to the growing gametophyte. Thus the gametophyte at maturity is differentiated into three zones, an upper fertile zone formed of large hyaline cells, a middle zone of small polygonal cells in an active state of division, and a basal haustorial zone. Viewed under a binocular microscope at this stage, the living teased-out gametophyte shows the upper fertile and translucent region with opaque areas marking the position of archegonia, while the two lower zones are opaque.

Abnormalities

Occasionally, for some unknown reason walls are not laid down in certain areas in some gametophytes at the free nucleate stage. Such areas remain multi-nucleate, or in them at a subsequent period a few walls may be laid down without any regularity. These areas markedly contrast with the rest of the homogeneously developed tissue of the female gametophyte, though they are apparently without significance.

In Pl. VI, Fig. 1, is illustrated a section of a very interesting embryo-sac in the free nucleate stage with nuclei in the process of division. This embryo-sac displayed polarity, the nuclei towards the micropylar end being at telophase while those lower down are at anaphase. Besides the normal nuclei in the process of division one large and highly polyploid nucleus also in a state of division is observed at A. This is lagging behind, the very numerous chromosomes being still at metaphase. Each chromosome is yet double—the daughters having not yet separated. Another section from the same gametophyte showed another nucleus at early telophase which also appeared polyploid. What the fate of such an embryo-sac would be it is difficult to say, but there are indications that it would degenerate.

DIPLOID FEMALE GAMETOPHYTES

While going through the slides it was observed that in certain cases the two ovules belonging to the same cone possessed striking differences in the size of the cells and nuclei of their gametophytes. Not only was there a marked difference in their cell size and nuclear size, but there was also a marked contrast in the size of their respective archegonia. This raised a suspicion that probably the gametophytes with larger cells and nuclei were of polyploid nature. The case was investigated further, and an attempt was made to secure earlier free-nucleate stages of the embryo-sacs with nuclei in the process of division to determine if really polyploid embryo-sacs arise in the species besides the normal ones.

In Pl. VI, Fig. 2, is shown a part of a normal gametophyte at free nucleate stage with nuclei at metaphase. In Pl. VI, Fig. 3, another gametophyte is observed with the nuclei at approximately the same stage of division embedded in the peripheral lining of the cytoplasm. Both of these belong to *Ephedra intermedia.* In none of these can the chromosomes be accurately counted, but the chromosome mass in the latter case appears double that of the former. Obviously the two gametophytes have a different chromosome constitution. The latter appear to be diploid. The formation of such probable diploid female gametophytes is not widespread in the species. They arise only sporadically, while the normal feature is the formation of haploid female gametophytes. Their occurrence, however, is of profound interest in view of the fact that diploid pollen grains to the extent of 2–5 per cent. have previously been recorded by the author in the present species as well as in some other species of the genus, like *E. saxatilis*, *E. altissima*, and *E. foliata* (Mehra, 1946). The method of formation of diploid pollen grains has also been traced in the case of *E. saxatilis* (Mehra, 1947). It has been observed that these diploid pollen grains germinate normally and produce diploid male gametophytes which in turn produce diploid male gametes.

Since the occurrence of diploid pollen grains is fairly widespread in the genus, a similar formation of diploid female gametophytes may be expected to be present in other species of the genus. It is necessary to section a large number of ovules before their presence can be detected.

DISCUSSION

Polymorphism in sex

It is interesting to note that this species exhibits polymorphism in sex. Some plants are either strictly male or strictly female, while others are hermaphrodite bearing male, female, and bisexual cones.

A chromosome study of the species has revealed it to be a tetraploid possessing 28 as the $2x$-chromosome number. It has been shown to contain 4 basic sets of 7 chromosomes each (Mehra, 1947).

Polyploids which have evolved from simpler unisexual diploids in which sex is determined by sex chromosomes are now known to display polymorphism in sex (similar to that exhibited by the present species) depending upon the ratio of X chromosomes to autosomes. The occurrence of sex chromosomes has not yet been established in any species of the genus *Ephedra* by cytological analysis, but the strict sexuality displayed by the male and female plants and the occurrence of hermaphrodite ones in individuals of the present species suggests that these individuals may be sex-controlled by the ratio of X chromosome to autosomes in their nuclear constitution. The species may have come from *immediate* unisexual diploid ancestors in which sex was governed by sex chromosomes. It seems that the sex chromosomes are not strikingly different morphologically from the autosomes in the genus *Ephedra*.

Hermaphrodite flowers and their significance

The three genera of the Gnetales, *Ephedra*, *Welwitschia*, and *Gnetum*, show characters in common, and yet there are striking differences in a number of morphologic and embryologic characters. Because of these differences they have been placed in three distinct families, namely, *Ephedraceae*, *Welwitschiaceae*, and the *Gnetaceae*.

One of these differences is in the structure of flowers. In *Ephedra* the flowers are typically unisexual. The male flower consists of two perianth leaves arranged anterio-posteriorly. Enclosed within is a central column-like structure, the sporangiophore, bearing at the apex a variable number of bilocular sporangia. The sporangiophore is supplied within by *two* vascular strands. The number of sporangia in many species is 8, although in others the number may be reduced to 3 or even 2. In *E. foliata* the number of sporangia is commonly 3. The sporangia may be sessile or in some species shortly stalked. Not infrequently the sporangiophore is bifurcate at the apex, the bifurcation sometimes extending very deep down. This has been noted by Thoday and Berridge (1912) in *E. distachya* and *E. nebrodensis* and by the writer in the present species. In such cases each shank is supplied with a single vascular strand. The total number of sporangia in these is evenly distributed on the two branches.

The structure of the male flower in *Gnetum* is on the same plan as in *Ephedra*. The perianth forms a cup-like envelope. From the centre of this projects an axial structure bearing two apical sporangia. Like *Ephedra*, this axial sporangiophore is traversed by *two* vascular strands.

In *Welwitschia*, on the other hand, the male flower is built very differently. It is pseudo-hermaphrodite. There are 4 perianth leaves, 2 outer smaller lateral, and 2 inner larger anterio-posterior. There are 6 stamens within, arranged in a ring. Each of these is supplied with a *single* vascular strand. These are united in the lower region to form a tube but are free above, each bearing terminally a trilocular sporangium. In the centre of the tube is a sterile ovule with its only integument produced above into a long micropylar tube characteristic of the Gnetales. In these sterile ovules the apex of the micropylar tube is flaring.

Apparently there seems to be a striking dissimilarity between the male flower of *Ephedra* or *Gnetum* on the one hand and that of *Welwitschia* on the other, and it has frequently been doubted if they are at all related.

The abnormal hermaphrodite flowers observed in *Ephedra intermedia* serve as a link connecting the normal male flower of *Ephedra* with that of *Welwitschia*. These abnormal flowers possess two perianth leaves arranged anterio-posteriorly as in the normal male flower. Enclosed within are two stamens bearing bilocular sporangia at their apices, while the centre is occupied by an ovule possessing a single integument drawn out above into a micropylar tube. Such a flower structure is remarkably similar in plan to that of *Welwitschia* except for certain differences of detail such as the number of perianth leaves and the number of stamens. These observations not only bring the Ephedraceae and the Welwitschiaceae close together but show that the modern Gnetales as a whole may have come from a stock with hermaphrodite flowers. In *Welwitschia*, relics of the ancestral condition are still retained with the pseudo-hermaphroditic condition of the male flower as a normal feature. Such a condition is normally lost in the Ephedraceae and the Gnetaceae, although, as in the present species, it may show itself exceptionally. The derivation of the male flower seen in the present-day forms of the genus *Ephedra* seems to have been from the original hermaphrodite flowers by the loss of the female part within and the fusion of two stamen stalks to form a central solid axial column. Thus the axial column is supplied by two vascular strands, whereas each stamen in *Welwitschia* possesses a single vascular strand. Also often the sporangiophore is more or less deeply cleft into its component parts. Sporangiophores which are bifurcated at the apex obviously represent a primitive condition as suggested by Thoday and Berridge (1912).

Nature of the outer integument of the ovule

The ovule in *Ephedra* possesses two integuments, an outer which is thick and fleshy and an inner thin membranous produced above into a long micropylar tube. The outer integument is free from the inner. In *E. intermedia* it is supplied by two vascular strands which are laterally disposed. The inner integument, on the other hand, is free from vascular supply and is fused with the nucellus excepting towards its micropylar end.

The morphological nature of the outer integument has been a matter of some discussion in the past. Certain abnormal and rather weakly developed ovules described and illustrated in the text suggested to the writer that the morphological nature of the outer integument is that of fused perianth leaves. The so-called outer integument in these ovules was thin and looked very much like the perianth of the male flowers. At its apex there were two distinct lobes which suggested that the outer integument is formed by the fusion of two 'leaf-like' structures. In another case the fusion between the two 'leaves' was completely absent on one side, while on the opposite side bifid nature was evident. That the outer integument is not a part of the ovule proper is further shown by the fact that it is entirely absent in the ovules present in the centre of the hermaphrodite flowers observed in *E. intermedia.*

Flower structure of the probable ancestors of the Gnetales

The flower in the ancestral stock of the Gnetales probably possessed two whorls of perianth leaves with two members in each whorl, a variable number of stamens (whose stalk was supplied with a single vascular strand) arranged in a ring bearing sporangia at the apices, and in the centre an ovule possessing a single integument which was fused with the nucellus in the lower region and was elongated above to form a micropylar tube. From this ancestral hermaphrodite flower by further modifications flower structure in *Welwitschia, Ephedra,* and *Gnetum* seems to have been derived along the following lines.

In *Welwitschia* the male flower has retained almost the original condition excepting for the fact that the ovule has become sterile—a trend towards the direction of unisexuality. The female flower is derived by the loss of inner anterio-posterior whorl of perianth leaves and the stamen ring. The two perianth leaves of the outer lateral whorl have united along their margins to form the outer envelop of the female flower.

In *Ephedra* in the derivation of the male flower the outer lateral whorl of perianth leaves has disappeared along with the ovule in the centre. The two anterio-posteriorly arranged members of the inner whorl have remained. At the same time the two stamens have undergone concrescence along the sides, giving rise to an axial column-like structure. This is supported by the fact that the stamen stalk of the present-day flowers is supplied by two vascular strands, while each stamen in *Welwitschia* possesses a single vascular bundle. In the female flower the inner anterio-posterior whorl of perianth leaves together with the two stamens have disappeared. The two lateral perianth leaves have fused along their margins, giving rise to the so-called outer integument as in *Welwitschia.*

In *Gnetum* the derivation of the male flower is on similar lines to that in *Ephedra.* In the female flower, on the other hand, the members of the outer whorl of perianth have coalesced to form the outermost envelope and the members of the inner whorl have united to form the middle envelope around the ovule. The ovule, strictly speaking, possesses only one integument which forms the micropylar tube. This has occurred together with the disappearance of the staminal whorl.

SUMMARY

Sex polymorphism is described in *Ephedra intermedia.* Some plants are strictly male or strictly female, while others are hermaphrodite, bearing male, female, and bisexual cones. Since *E. intermedia* is tetraploid it is suggested that display of sex may be governed in the individuals by the ratio between X chromosome and autosomes as is the case in other polyploids derived from unisexual diploids.

The hermaphrodite flowers are built on the same plan as the pseudo-hermaphrodite male flowers of *Welwitschia*. There are two perianth leaves arranged in a median plane followed inwards by two stamens each bearing three or four bilocular sporangia with an ovule in the centre possessing single integument produced upwards into a micropylar tube. The significance of this discovery is discussed.

The archesporial cell in *E. intermedia* and *E. saxatilis* is hypodermal in origin and becomes directly transformed into a megaspore mother-cell. The usual linear tetrad of megaspores is formed although sometimes the upper diad does not divide. The lowest megaspore is functional.

The development of the embryo-sac follows the general plan in this genus, although some variation is observed in the time of wall formation and in the occurrence of diploid or polyploid nuclei.

Occasionally diploid female gametophytes appear besides the normal haploid ones.

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EXPLANATION OF PLATES

Illustrating P. N. Mehra's article 'Occurrence of Hermaphrodite Flowers and the Development of Female Gametophyte in *Ephedra intermedia* Shrenk et Mey.'

PLATE V

Fig. 1. Cones from a monoecious plant of *Ephedra intermedia*. (× about 2½.) *a*. Bisexual cone. *b*. A cluster of two cones, terminal female and lateral male. *c*. A cluster of two cones, terminal female and lateral bisexual. *d*. A cluster of three cones, terminal female and lateral male, and the other bisexual.

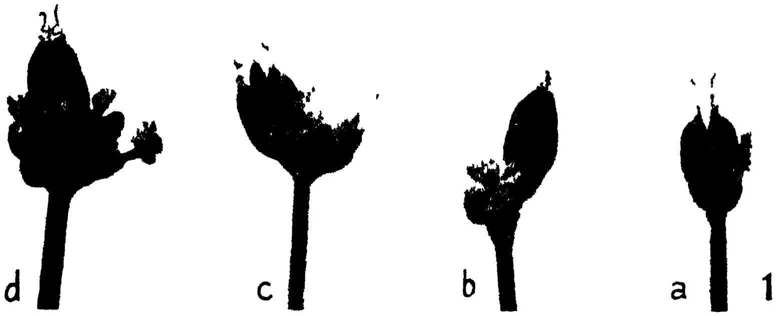
Fig. 2. V.S. through a hermaphrodite flower of *E. intermedia*. Note the ovule in the centre with its only integument elongated above to form a micropylar tube T. Outside it on the right a stamen is visible bearing a bilocular sporangium. The stalk of the second stamen is in the succeeding section, but one of its sporangium is visible. Outside are the two perianth leaves marked PP.

PLATE VI

Fig. 1. Section through free-nucleate embryo-sac with nuclei in division stage. There is polarity; those towards the upper micropylar end are at telophase while chalazal ones are at anaphase. At A is observed an exceptional highly polyploid nucleus at metaphase with a very large number of chromosomes.

Fig. 2. Section through a normal haploid embryo-sac at free-nucleate stage with nuclei at metaphase.

Fig. 3. Section through another embryo-sac at free-nucleate stage with nuclei at the same stage as in the previous figure. The metaphase clump here is double that in the former. This is probably diploid.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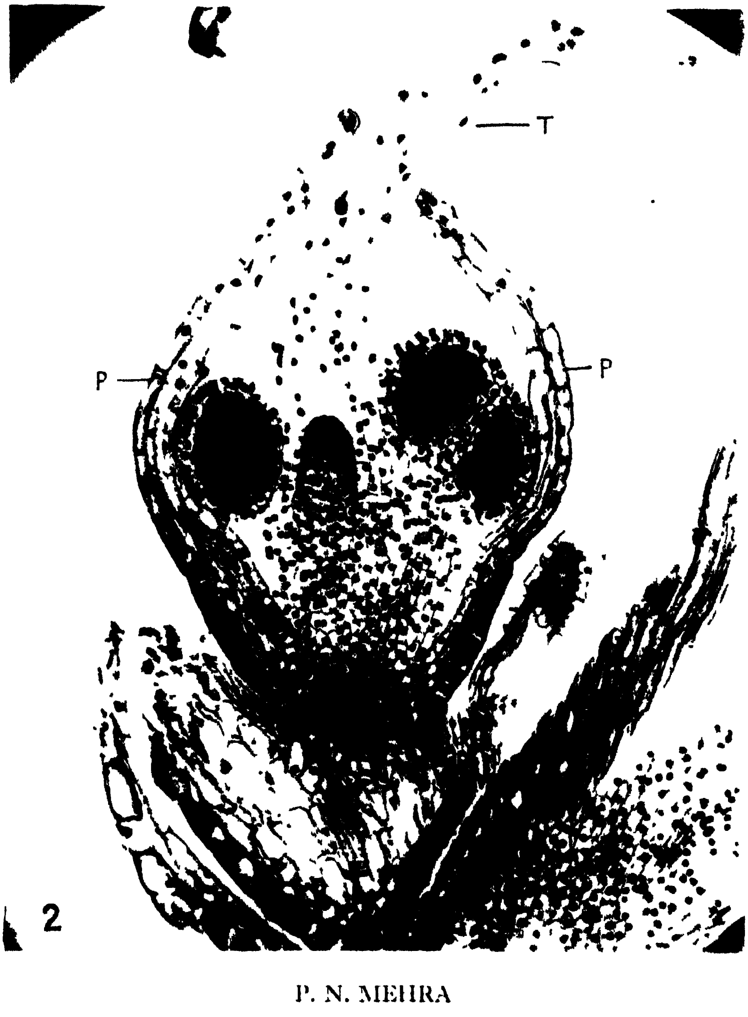
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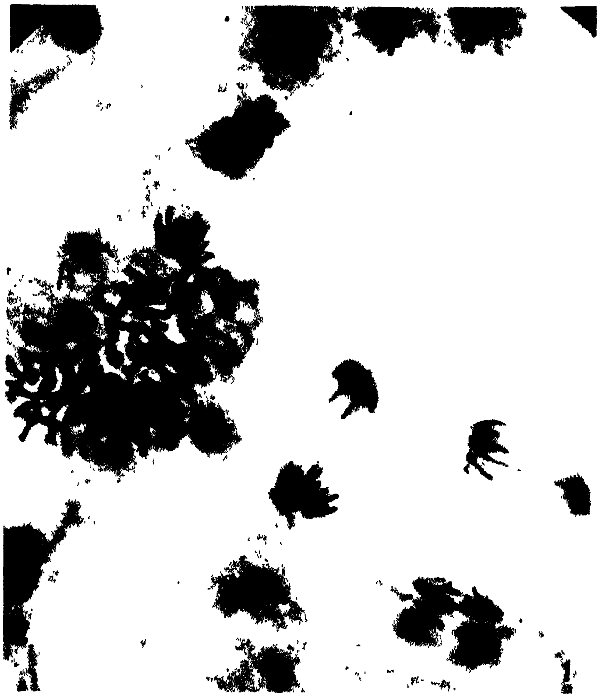
P. N. MEHRA



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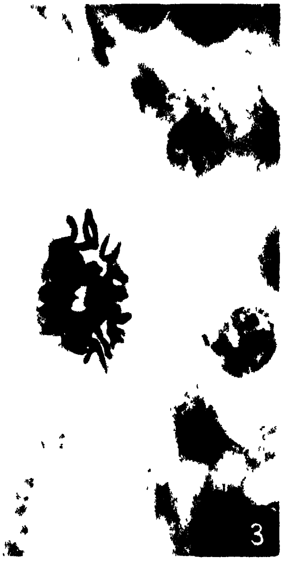

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P. N. MEHRA

The Effect of the Plant Growth Regulator, 4-Chloro 2-Methyl Phenoxyacetic Acid, on the Mineral and Nitrogen Contents of Plants

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INTRODUCTION

TEMPLEMAN and Sexton (1946) have shown that certain concentrations of the aryloxyacetic acids, notably 4-chloro 2-methyl phenoxyacetic acid (M.C.P.A.), produce a differential effect on the growth of different species and that this may be utilized as a means of selective weed control.

Rhodes and Templeman (1947) investigated the nature of this differential response in spring oats (variety 'Victory'), corn chamomile (*Anthemis arvensis*), and rape (*Brassica campestris*), and reported that the sodium salt of 4-chloro 2-methyl phenoxyacetic acid markedly interfered with the potassium metabolism of the most susceptible species, rape, while having little or no effect on that of corn chamomile and oats. In the present paper these preliminary results are presented in greater detail together with a further investigation of the effect of sodium 4-chloro 2-methyl phenoxyacetate on the mineral and nitrogen contents of another susceptible species, tomato (*Solanum lycopersicum*), and the influence of the potassium status of the plant on its response.

PART I

The Effect of Sodium 4-Chloro 2-Methyl Phenoxyacetate on the Mineral Content and Growth of Species having Varying Susceptibilities to Its Action

Experimental details

As a result of field experience, rape and spring oats were selected as examples of susceptible and resistant crops, and corn chamomile for intermediate response.

Seedlings of the three species were grown from seed in small pots containing silver sand. Two hundred pots for each species were sown, the number of seeds per pot varying with the size of seed in the following manner:

Rape (*Brassica campestris*)	16 seeds per pot
Corn chamomile (*Anthemis arvensis*) . . .	approximately 30 seeds per pot
Spring oats (var. 'Victory')	12 seeds per pot

[Annals of Botany, N.S. Vol. XIV, No. 54, April, 1950.]

The pots were lightly watered daily until the seedlings were showing above the sand and thereafter 40 ml. of nutrient solution was applied per pot, once or twice daily according to weather conditions, the nutrient containing 100 p.p.m. N, 50 p.p.m. P_2O_5, 200 p.p.m. K_2O, 40 p.p.m. MgO, together with other essential elements in lower concentrations, and having a pH of 6.

When the seedlings were well established they were thinned to four seedlings per pot and, for each species, 150 of the most uniform pots were selected for experimentation.

In each species the pots were allotted at random into five sets, A, B, C, D, and E, each consisting of 30 pots. The five sets from a given species were placed in five parallel rows on a trolley so that they could be moved as required. Five treatment solutions, the highest of which was estimated to be the maximum concentration which the most susceptible species could survive, were prepared by dilution with the nutrient solution from a stock solution of 0·01 g. per cent. of the sodium salt of M.C.P.A. and allotted to the sets of pots as follows:

A . . .	0 p.p.m.	sodium	4-chloro 2-methyl	phenoxyacetate
B . . .	0·01 p.p.m.	,,	,,	,,
C . . .	0·05 p.p.m.	,,	,,	,,
D . . .	0·10 p.p.m.	,,	,,	,,
E . . .	0·50 p.p.m.	,,	,,	,,

From the time when the seedlings were 3 to 4 weeks old the treatment solutions were applied from a graduated separating funnel to the sand surface of the appropriate pots at the rate of 40 ml. per pot either once or twice daily according to weather conditions. Application of the treatments began on the following dates:

Rape	May 9, 1946
Corn chamomile	May 11, 1946
Oats	May 1, 1946

At approximately weekly intervals one sample consisting of five pots was withdrawn at random from each treatment within a given species. The roots were washed free from sand, blotted to remove surface moisture, and separated from the 'tops'. The samples were then killed and dried at approximately 100° C. and the dry weights of the samples from five plants were determined. In some samples (p. 188) a further subdivision of 'tops' was made.

The dried 'tops' from the five plants taken from each species at a given sampling time were then bulked, ground to a powder in a Christie & Norris 5-in. mill, re-dried at 100° C., and taken for determination of percentage compositions in regard to potassium, phosphorus, and magnesium. The estimations of magnesium and potassium were done spectrographically, while the phosphorus was determined chemically. Details of the analytical methods are given in the appendix.

Owing to the amount of work involved it was not possible to examine all samples from each species, but the mineral determinations were made on the more important ones, namely, 0, 0·1, and 0·5 p.p.m. M.C.P.A.

Results

i. *Rape* (*Brassica campestris*). This species was highly susceptible. Chlorosis of the foliage, swelling of the hypocotyl and root-tips, presence of adventitious roots, and a less fibrous appearance of the roots were marked after only 7 days' treatment at 0·5 p.p.m. A similar effect was noticeable on the roots after 35 days with the concentration so low as 0·05 p.p.m. After 5 weeks' treatment at 0·5 p.p.m., the root system consisted almost entirely of bulbous root initials which had arisen from the main root.

ii. *Corn chamomile* (*Anthemis arvensis*). This species was moderately susceptible. Characteristic changes in the morphology of the roots and hypocotyl appeared after 17 days of treatment at 0·5 p.p.m. There was a marked development of adventitious roots just behind the apices of the lateral roots, these adventitious roots, being in one plane, giving a feather-like appearance to the parent root.

iii. *Spring oats* (*variety 'Victory'*). This species was most resistant. The roots were unaffected until 28 days after beginning the 0·5 p.p.m. treatment, when it was noted that the adventitious roots which had developed near the apices of the main roots were very short and had bulbous swellings at their apices.

The mean dry weight per plant of the roots and 'tops' at each sampling time are tabulated in Table I, while the K_2O, P_2O_5, and MgO contents of the bulked samples of 'tops' expressed as a percentage of the bulked dry weights of the 'tops' are given in Table II.

Discussion

The visual observations show quite clearly the differential response of the three species to sodium 4-chloro 2-methyl phenoxyacetate. For example, after only 7 days of treatment there was an effect on the root system of rape at 0·5 p.p.m., but for corn chamomile this was not observed until 17 days and for oats 28 days. Again, rape was the only species to show thickening of the lateral roots at 0·05 p.p.m.

The dry-weight figures also bring out the differences in species sensitivity. Thus for the 'tops' there is a significant reduction at 0·5 p.p.m. for rape at all sampling times after the first, while there is also a significant reduction at 0·1 p.p.m. at the sixth sampling time. For corn chamomile similar reductions are observed at 0·5 p.p.m., but the differences are much smaller and there is no effect at 0·1 p.p.m. For oats there is no reduction in dry weight at any sampling time, even at the highest concentration of M.C.P.A. (Actually there is a just significant reduction at 0·5 p.p.m. at the third sampling time, but since there is no indication of such reduction at any subsequent sampling time it would appear to be fortuitous.)

For the roots, only rape is affected at 0·5 p.p.m., differences being found at the third and later sampling times. It is particularly interesting to note that the dry weight of the 'tops' are reduced before any reduction in root dry weight is observed, although, as previously described, the morphology of the root has already been modified.

TABLE I

Mean Dry Weight per Plant—'Tops' and Roots

'TOPS'

Sampling time.	Period of treat ment (days).	Mean dry weight per plant.					Standard error of singl sample.	Coefficients o variation.	Significant difference be-tween means.
		0 p.p.m. M.C.P.A.	0·01 p.p.m. M.C.P.A.	0·05 p.p.m. M.C.P.A.	0·10 p.p.m. M.C.P.A.	0·5 p.p.m. M.C.P.A.			
Rape (seed sown April 15)								%	
May 17	7	0·09	0·09	0·10	0·10	0·12	0·03	29·1	N.S.
„ 24	14	0·28	0·26	0·28	0·27	0·12	0·06	24·6	0·08
„ 31	21	0·57	0·64	0·57	0·57	0·15	0·10	20·1	0·13
June 7	28	0·70	0·71	0·73	0·62	0·18	0·10	17·0	0·13
„ 14	35	1·33	1·02	1·14	1·11	0·21	0·19	19·6	0·25
„ 21	42	1·64	1·30	1·54	1·07	0·32	0·20	17·1	0·26
Corn Chamomile (seed sown April 17)									
May 21	10	0·04	0·04	0·03	0·04	0·04	0·01	35·2	N.S.
„ 28	17	0·08	0·08	0·09	0·07	0·02	0·03	47·3	0·04
June 4	24	0·26	0·33	0·32	0·29	0·15	0·07	27·7	0·10
„ 11	31	0·52	0·50	0·54	0·57	0·32	0·12	23·6	0·15
„ 19	39	0·90	0·68	0·84	0·76	0·57	0·21	27·6	N.S.
„ 25	45	1·12	0·99	1·13	0·93	0·58	0·22	23·2	0·29
Oats (seed sown April 17)									
May 8	7	0·16	0·15	0·11	0·15	0·12	0·04	30·3	N.S.
„ 15	14	0·15	0·15	0·18	0·19	0·23	0·05	28·3	N.S.
„ 21	20	0·37	0·33	0·39	0·34	0·26	0·05	14·4	0·06
„ 29	28	0·70	0·71	0·68	0·69	0·66	0·13	18·4	N.S.
June 5	35	1·16	1·08	1·17	1·08	1·06	0·15	13·2	N.S
„ 13	43	2·11	1·75	2·01	2·09	1·70	0·25	13·0	N.S.

ROOTS

Sampling time.	Period of treat ment (days).	0 p.p.m. M.C.P.A.	0·01 p.p.m. M.C.P.A.	0·05 p.p.m. M.C.P.A.	0·10 p.p.m. M.C.P.A.	0·5 p.p.m. M.C.P.A.	Standard error of singl sample.	Coefficients o variation.	Significant difference be-tween means.
Rape (seed sown April 15)									
May 17	7	0·06	0·06	0·04	0·05	0·05	0·02	41·4	N.S.
„ 24	14	0·07	0·05	0·06	0·07	0·06	0·02	26·5	N.S.
„ 31	21	0·11	0·11	0·11	0·15	0·10	0·06	48·6	N.S.
June 7	28	0·19	0·19	0·19	0·14	0·08	0·05	34·6	0·07
„ 14	35	0·39	0·33	0·30	0·38	0·14	0·07	23·3	0·10
„ 21	42	0·45	0·38	0·49	0·39	0·16	0·12	33·8	0·17
Corn Chamomile (seed sown April 17)									
May 21	10	0·02	0·02	0·01	0·02	0·01	0·01	29·5	N.S.
„ 28	17	0·04	0·04	0·05	0·05	0·04	0·02	57·0	N.S.
June 4	24	0·09	0·12	0·13	0·12	0·09	0·03	31·4	N.S.
„ 11	31	0·15	0·14	0·18	0·22	0·11	0·05	33·9	N.S.
„ 19	39	0·25	0·20	0·21	0·22	0·20	0·06	28·2	N.S.
„ 25	45	0·24	0·18	0·26	0·20	0·18	0·02	11·6	N.S.
Oats (seed sown April 17)									
May 8	7	0·16	0·17	0·23	0·10	0·16	0·07	42·4	N.S.
„ 15	14	0·16	0·18	0·18	0·13	0·20	0·05	26·9	N.S.
„ 21	20	0·22	0·20	0·21	0·19	0·30	0·06	25·0	0·08
„ 29	28	0·29	0·33	0·34	0·33	0·45	0·07	22·1	N.S.
June 5	35	0·55	0·50	0·48	0·61	0·80	0·17	28·2	N.S.
„ 13	43	0·71	0·70	0·76	0·74	0·77	0·11	15·0	N.S.

N.S. = Not significant.

TABLE II

K_2O, P_2O_5, and MgO Contents of Bulked Samples of 'Tops' expressed as Percentage of the Dry Weights

Sampling time.	Dry weight of bulked sample (g.)			K_2O content as % of bulked dry weight			P_2O_5 content as % of bulked dry weight			MgO content as % of bulked dry weight		
				A	D	E	A	D	E	A	D	E
	0 p.p.m. M.C.P.A.	0·1 p.p.m. M.C.P.A.	0·5 p.p.m. M.C.P.A.	0 p.p.m. M.C.P.A.	0·1 p.p.m. M.C.P.A.	0·5 p.p.m. M.C.P.A.	0 p.p.m. M.C.P.A.	0·1 p.p.m. M.C.P.A.	0·5 p.p.m. M.C.P.A.	0 p.p.m. M.C.P.A.	0·1 p.p.m. M.C.P.A.	0·5 p.p.m. M.C.P.A.
Rape (seed sown April 15)												
May 17	0·47	0·52	0·61	3·0	2·8	2·5	1·9	—	1·6	0·9	0·7	0·5
„ 24	1·61	1·33	0·60	5·8	5·9	4·5	1·5	1·5	1·4	0·8	0·8	0·8
„ 31	2·87	2·84	0·73	7·2	7·2	2·5	1·0	1·2	1·0	0·8	0·9	1·1
June 7	3·48	3·08	0·92	12·3	10·0	4·8	1·1	1·4	1·3	2·1	2·8	6·3
„ 14	6·65	5·57	1·06	10·0	10·0	3·4	1·3	1·3	1·1	1·3	1·6	2·6
„ 21	8·22	5·36	1·60	9·2	8·5	3·6	1·1	1·1	1·1	1·0	1·7	2·6
Corn chamomile (seed sown April 17)												
May 21	0·22	0·20	0·19	11·8	11·1	12·2	—	—	—	0·9	1·0	1·1
„ 28	0·40	0·36	0·12	12·9	11·6	10·6	1·8	1·7	—	0·8	0·7	1·5
June 4	1·30	1·43	0·74	10·6	11·1	8·4	1·2	1·3	1·4	0·7	0·7	0·9
„ 11	2·60	2·67	1·59	10·8	6·0	6·0	1·5	1·5	1·6	0·9	0·9	1·2
„ 19	4·50	3·80	2·87	8·7	10·2	9·2	1·2	1·2	1·2	0·9	1·1	1·1
„ 25	5·62	4·67	2·91	8·7	9·2	7·6	0·8	1·0	1·1	1·1	1·1	1·0
Oats (seed sown April 17)												
May 8	0·80	0·75	0·58	9·4	7·2	7·1	2·4	2·3	2·3	0·5	0·7	0·6
„ 15	0·77	0·95	1·16	7·1	5·6	4·8	2·4	2·2	2·0	0·6	0·5	0·4
„ 21	1·85	1·69	1·30	7·0	6·0	5·6	1·9	1·9	2·0	0·7	0·6	0·5
„ 29	3·50	3·42	3·28	8·6	8·1	6·0	1·8	1·4	1·4	0·6	0·6	0·6
June 5	5·82	5·42	5·29	6·1	6·9	5·9	1·6	1·4	1·4	0·4	0·5	0·5
„ 13	10·50	10·44	8·45	7·4	8·3	5·0	1·3	1·4	1·4	0·4	0·4	0·5

The fact that the above differential responses were produced by applications of M.C.P.A. to the roots only clearly indicates that they are due to physiological differences between the three species and not to any differences in morphology of the aerial portions.

From the data of Table II it is seen that there are no differences due to M.C.P.A. treatment in P_2O_5 content of the tops of any of the three species. A very different picture is seen for the K_2O contents, where there are marked reductions in K_2O content of the rape 'tops' at 0·5 p.p.m. at all sampling times. No such obvious or consistent differences are observed for the other two species. Since rape also showed the greatest depression in dry weight of the tops, it is clear that the reduction in K_2O content of the rape tops is even greater than the percentage figures of Table II would suggest.

For magnesium content there is obviously no depression corresponding to that found for potash in any species. If anything, there appears to be a greater MgO content of the rape 'tops' at 0·1 p.p.m. and 0·5 p.p.m. M.C.P.A. at the fourth and subsequent sampling times.

Rape, the most susceptible species, thus appears to differ from the other species in two ways:

(i) Examination of the control figures in Table II shows that the K_2O content of the tops of corn chamomile and of oats does not vary much throughout the period of treatment, although in the case of the former the dry weight increased by about 27 times and in the case of the latter by about 10 times. The K_2O content of the untreated rape, on the other hand, rose gradually during the first 28 days with increasing dry weight and then remained roughly the same although the dry weight continued to increase.

(ii) Treatment of rape with 0·5 p.p.m. M.C.P.A., besides giving a considerable reduction of dry-matter production, has also retained the K_2O content at about the low figure of the first sample right throughout the period of treatment.

Thus it is seen that the sodium salt of 4-chloro 2-methyl phenoxyacetic acid markedly interferes with the potassium metabolism of the most susceptible species. The nature of this interference and its possible bearing on the causes of the selective phytocidal action are further investigated in Part 2.

PART 2

The Effect of 4-Chloro 2-Methyl Phenoxyacetic Acid on the Mineral and Nitrogen Contents of a Susceptible Species, Solanum Lycopersicum (Tomato)

The specific depression of the potash content of the tops of rape described in Part 1 was followed by a more extensive examination of the effect of M.C.P.A. on the mineral and nitrogen contents of another susceptible species, *Solanum lycopersicum* (tomato), and the influence of the external potassium supply on the response.

Experimental details

On September 2, 1947, 100 uniform seedling tomatoes (variety 'Stonor's M.P.') were selected from a group of 3-week-old plants which had been grown in sand under normal soil-less culture conditions in an unheated glasshouse. These plants were potted into 48-size pots with silver sand and allotted into five blocks of 20 plants each. Within each block all treatments were distributed to the plants at random. Owing to limitations of space and the necessity for employing non-technical assistance in the daily application of the treatments, no randomization of the position of plants within a block was possible. The results, therefore, have not been submitted to statistical analysis, but they have been interpreted on the basis of large differences shown by the treatment means.

For the following 17 days the plants in each block were given five nutrient solution treatments in which the ratio of K_2O/Na_2O was varied as follows:

K(1) . . .	37 p.p.m. K_2O	606 p.p.m. Na_2O
K(2) . . .	67 ,,	566 ,,
K(3) . . .	117 ,,	496 ,,
K(4) . . .	217 ,,	371 ,,
K(5) . . .	372 ,,	206 ,,

These levels of potassium and sodium were selected so as to maintain, as far as possible, a constant total salt concentration for all nutrient solutions, while leaving the composition unaltered with respect to other ions. Of the amounts of sodium and potassium supplied, 206 p.p.m. Na_2O and 17 p.p.m. K_2O were present in the main water supply.

In preparing the different nutrient solutions, a basal stock solution was made up for subsequent dilution at 1 in 100. This solution contained:

$MgSO_4 . 7H_2O$	246 g.
$NH_4H_2PO_4$	81 g.
K_2SO_4	37 g.
Minor elements concentrate (1)[1]	1 litre
Distilled water to 10 litres.	

50 ml. of the stock solution were diluted with 4·75 litres of tap-water containing one of the following mixtures of potassium nitrate and sodium nitrate to give the treatment solutions K(1)-K(5) which were adjusted to pH 6 by the addition of sulphuric acid as required:

For K(1)	2·735 g. $NaNO_3$ . . .	Nil KNO_3
,, K(2)	2·460 g. ,, . . .	0·320 g. ,,
,, K(3)	2·000 g. ,, . . .	0·855 g. ,,
,, K(4)	1·125 g. ,, . . .	1·920 g. ,,
,, K(5)	Nil ,, . . .	3·600 g. ,,

Two applications, each of 50 ml., were given daily per pot.

---

[1] The Minor elements concentrate (1) contained the following salts dissolved in 10 litres of distilled water:

Lithium chloride ($LiCl \cdot 2H_2O$) .	0·28 g.	Nickel sulphate ($NiSO_4$ $6H_2O$) .	0·56 g.
Copper sulphate ($CuSO_4 \cdot 5H_2O$) .	0·56 ,,	Cobalt nitrate ($Co(NO_3)_2 \cdot 6H_2O$) .	0·56 ,,
Zinc sulphate ($ZnSO_4 \cdot 7H_2O$) .	0·56 ,,	Titanium oxide (TiO_2) .	0·56 ,,
Boric acid (H_3BO_3) . . .	6·12 ,,	Potassium iodide (KI) . .	0·28 ,,
Aluminium sulphate ($Al_2(SO_4)_3$) .	0·56 ,,	Potassium bromide (KBr) . .	0·28 ,,
Stannous chloride ($SnCl_2 \cdot 2H_2O$).	0·28 ,,	Sodium silicate (Na_2SiO_3) . .	4·33 ,,
Manganese sulphate ($MnSO_4 \cdot 4H_2O$)	0·75 ,,	Ferric citrate ($FeC_6H_5O_7$ $3H_2O$) .	64·20 ,,

On September 19, after 17 days of the above treatments, there were no visible differences. The sodium 4-chloro 2-methyl phenoxyacetate treatments were then superimposed on the potassium-level treatments to give the following concentrations of M.C.P.A. in the nutrient solutions:

(*a*) 0 p.p.m. sodium 4-chloro 2-methyl phenoxyacetate.
(*b*) 0·1 p.p.m. ,, ,, ,, ,,
(*c*) 0·3 p.p.m. ,, ,, ,, ,,
(*d*) 0·9 p.p.m. ,, ,, ,, ,,

From September 30 until the completion of the experiment, one daily application of 90 ml. of nutrient was applied per pot.

By October 2 the plants had grown considerably and the concentration of M.C.P.A. was therefore increased to the following levels:

(*a*) 0 p.p.m. sodium 4-chloro 2-methyl phenoxyacetate.
(*b*) 0·15 p.p.m. ,, ,, ,, ,,
(*c*) 0·45 p.p.m. ,, ,, ,, ,,
(*d*) 1·35 p.p.m. ,, ,, ,, ,,

The plants were subjected to the M.C.P.A. treatments for a total period of 21 days, during which time visual observations were made. At the end of this period, on October 10, the plants were harvested. Each plant was divided into three portions, 'root', 'lower stem', and 'tops', obtained in the following manner:

'Root': Stem cut at insertion of cotyledons. Roots carefully washed free from sand and blotted dry.

'Lower stem': A second cut was made in the stem at a point 7 in. above the insertion of the cotyledons. Since the lowest leaves on this portion were rather damaged owing to the surface application of nutrient, all the leaves (a maximum of three) were removed from the lower stem and discarded.

'Tops': This portion comprised all the stem and leaves above the 'lower stem' portion.

The tissues were killed in an oven at 95° C. and the dry weights were then determined by drying at 90° C.

The dried samples were ground to a powder in a Christie & Norris 5-in. mill and then placed in 4-oz. screw-cap jars until required for analysis.

Details of the analytical methods are given in the appendix.

Results

(*a*) *Visual observations*

19/9/47. Commenced to apply M.C.P.A. treatments.
24/9/47. Lower leaves of all plants receiving the highest level (*d*) of M.C.P.A. showed epinasty in the region of the pulvinus.
1/10/47. Lower leaves of all plants receiving M.C.P.A. treatment (*c*) showed similar effects to those previously observed for treatment (*d*). The leaves in these two treatments appeared somewhat 'emaciated' and the lower leaves were yellowish.

In treatment (*d*) the plants receiving the three lowest potash levels, K(1), K(2), and K(3), had young leaves which showed a slight but definite chlorosis of the leaf margins.

Adventitious root initials were apparent as bulges on the lower 4 in. of stem of all plants receiving treatment (*d*).

2/10/47. First truss just visible on all plants.

8/10/47. Opening of flowers of first truss delayed in plants receiving highest M.C.P.A. treatment. Thus only 3 out of 20 plants had open flowers in treatment (*d*) as against 17, 16, and 16 for treatments (*a*), (*b*), and (*c*) respectively.

Growth of the axillary buds was progressively inhibited with increasing concentration of M.C.P.A. Thus the shoot in the axil of the leaf immediately below the first truss measured as follows:

M.C.P.A. treatment (*a*) 3–4 in. in length.
,, ,, (*b*) 1–3 in. in length.
,, ,, (*c*) less than 1 in. in length.
,, ,, (*d*) No axillary shoot.

The plants receiving M.C.P.A. treatments (*b*), (*c*), and (*d*) appeared 'spindly', this effect being more marked with increasing concentration of M.C.P.A.

Adventitious root initials were apparent as bulges on the stems of plants receiving M.C.P.A. treatment (*c*), similar to those previously noted for treatment (*d*).

10/10/47. All plants harvested.

(*b*) *Dry weights and analytical data.* For all treatments the mean values of the five replicates with respect to dry weight and total nitrogen, K_2O, P_2O_5, MgO, and CaO contents expressed as percentages of the dry weights are given in Table III. From these data the mean values of the absolute weights of total nitrogen, K_2O, P_2O_5, MgO, and CaO, have been calculated and these are presented in Table IV.

It is clear that, in general, the trends shown by increasing M.C.P.A. over all potash levels are similar, as are those shown by increasing potash over all M.C.P.A. levels. To facilitate interpretation, therefore, the data have been condensed in Table V, where the mean values of dry weight and total nitrogen, K_2O, P_2O_5, MgO, and CaO expressed as percentages of the dry weights, over all M.C.P.A. levels are shown for increasing levels of potash, together with the mean values over all potash levels for increasing M.C.P.A. concentrations.

The data of Table IV for the absolute amounts of total nitrogen, K_2O, P_2O_5, MgO, and CaO have been condensed in a similar manner in Table VI.

Visual examination of the spectrographic plates showed no consistent or obvious differences between treatments in iron, copper, manganese, or sodium in the tops or roots.

Discussion

The general effect of M.C.P.A. treatment on the mineral status of the tomato plant is very similar to that previously noted for another susceptible species, rape. Thus the data of Table III show that M.C.P.A. treatment results in a markedly lower K_2O content of the 'tops', this effect becoming progressively greater with increasing concentration of M.C.P.A. A similar condition is found for the 'lower stems'. In the roots, however, which were

TABLE III

Mean Values of Dry Weights and Total N, K_2O, P_2O_5, MgO, and CaO Contents expressed as Percentages of the Dry Weights

	K(1)				K(2)				K(3)				K(4)				K(5)			
	(a)	*(b)*	*(c)*	*(d)*	*(a)*	*(b)*	*(c)*	*(d)*	*(a)*	*(b)*	*(c)*	*(d)*	*(a)*	*(b)*	*(c)*	*(d)*	*(a)*	*(b)*	*(c)*	*(d)*
TOPS																				
Dry weight (g.)	1·67	1·74	1·46	0·74	1·80	1·64	1·52	0·93	2·08	1·80	1·53	1·05	2·22	1·95	1·48	1·02	2·47	2·35	1·57	0·97
Total N content (as % of dry wt.)	4·32	3·61	3·72	3·73	3·97	3·83	3·67	3·96	3·87	3·65	3·72	3·66	3·70	3·86	3·71	3·67	3·62	3·57	3·71	3·53
„ K_2O „ „ „	5·9	5·4	4·5	3·1	6·4	5·8	4·7	3·7	7·4	7·3	5·8	4·6	8·2	7·7	6·8	4·6	8·3	8·0	7·6	6·0
„ P_2O_5 „ „ „	1·36	1·38	1·35	1·29	1·38	1·38	1·30	1·12	1·48	1·37	1·30	1·06	1·39	1·27	1·15	1·17	1·36	1·22	1·13	0·99
„ MgO „ „ „	1·7	2·1	2·0	1·9	1·8	1·8	2·0	2·0	1·5	1·6	1·9	1·9	1·6	1·7	1·7	2·0	1·5	1·6	1·6	1·5
„ CaO „ „ „	1·1	1·3	1·3	1·2	1·4	1·3	1·3	1·5	1·2	1·2	1·2	1·3	1·1	1·1	1·5	1·2	1·1	1·3	1·0	1·0
LOWER STEMS																				
Dry weight (g.)	0·80	0·97	0·88	0·90	0·87	0·87	0·89	1·13	0·93	0·93	0·74	0·95	1·00	0·92	0·92	1·07	1·09	0·98	1·03	1·18
Total N content (as % of dry wt.)	1·97	1·69	1·60	1·58	1·48	1·52	1·67	1·55	1·50	1·69	1·43	1·53	1·62	1·57	1·29	1·53	1·55	1·48	1·43	1·54
„ K_2O „ „ „	4·5	2·8	2·9	2·4	5·2	4·9	3·3	3·0	6·6	5·5	4·9	3·8	7·3	6·7	5·8	6·0	7·8	7·3	7·7	6·9
„ P_2O_5 „ „ „	1·00	0·91	0·90	0·97	0·98	0·92	0·90	0·96	0·92	0·97	0·88	0·88	1·08	0·98	0·81	0·91	1·12	0·91	0·81	0·81
„ MgO „ „ „	1·5	1·7	1·6	1·8	1·4	1·4	1·5	1·5	1·3	1·3	1·5	1·4	1·2	1·2	1·2	1·2	1·0	1·2	1·1	0·9
„ CaO „ „ „	0·6	0·6	0·7	0·7	0·7	0·5	0·6	0·5	0·5	0·6	0·5	0·6	0·5	0·5	0·5	0·5	0·5	0·5	0·4	0·4
ROOTS																				
Dry weight (g.)	0·72	0·89	0·74	0·72	0·83	0·61	0·86	0·71	0·81	0·72	0·67	0·59	0·94	0·74	0·84	0·70	0·96	0·88	1·01	0·96
Total N content (as % of dry wt.)	2·40	2·18	2·56	2·94	2·92	2·23	2·42	3·04	2·25	2·38	2·76	3·00	2·18	2·09	2·58	3·46	1·99	2·38	2·76	3·56
„ K_2O „ „ „	2·2	2·2	2·8	4·1	1·9	2·7	3·1	4·4	2·5	3·2	4·2	4·9	3·2	3·8	4·6	5·6	4·3	4·9	5·7	6·4
„ P_2O_5 „ „ „	0·77	0·76	0·79	1·06	0·79	0·78	0·87	1·11	0·78	0·78	0·92	1·04	0·81	0·81	0·84	1·08	0·77	0·80	0·90	1·16
„ MgO „ „ „	1·1	1·2	0·9	0·9	1·2	1·0	0·9	0·9	1·2	1·0	0·9	0·8	1·0	0·9	0·9	0·8	1·0	0·8	0·8	0·7
„ CaO „ „ „	0·5	0·5	0·5	0·5	0·5	0·5	0·4	0·5	0·6	0·5	0·5	0·6	0·5	0·5	0·5	0·5	0·4	0·5	0·4	0·5

TABLE IV

Mean Values of Dry Weights and Absolute Amounts of Total N, K_2O, P_2O_5, MgO, and CaO

	K(1)				K(2)				K(3)				K(4)				K(5)			
	(a)	(b)	(c)	(d)	(a)	(b)	(c)	(d)	(a)	(b)	(c)	(d)	(a)	(b)	(c)	(d)	(a)	(b)	(c)	(d)
TOPS																				
Dry weight (g.)	1·67	1·74	1·46	0·74	1·80	1·64	1·52	0·93	2·08	1·80	1·53	1·05	2·22	1·95	1·48	1·02	2·47	2·35	1·57	0·97
Absolute amount total N (mg.)	70	62	54	27	71	63	55	37	80	65	57	38	82	74	54	37	80	82	58	34
,, ,, K_2O ,,	94	91	65	22	113	97	73	34	156	130	88	47	183	145	104	45	201	191	120	62
,, ,, P_2O_5 ,,	22	24	20	9	25	23	20	11	31	25	20	11	31	25	17	12	33	29	18	10
,, ,, MgO ,,	28	35	30	14	32	30	30	19	32	29	30	20	36	33	25	20	38	37	25	16
,, ,, CaO ,,	10	22	19	0	26	22	19	14	24	21	19	13	24	21	22	12	28	30	16	10
LOWER STEMS																				
Dry weight (g.)	0·80	0·97	0·88	0·60	0·87	0·87	0·89	1·13	0·93	0·93	0·74	0·95	1·00	0·92	0·92	1·07	1·09	0·98	1·03	1·18
Absolute amount total N (mg.)	15	16	14	14	16	13	13	17	14	16	11	14	16	14	12	17	17	15	15	18
,, ,, K_2O ,,	37	20	25	21	44	44	29	33	62	52	35	35	74	61	55	64	84	71	78	79
,, ,, P_2O_5 ,,	8	9	8	9	9	8	8	11	9	9	7	8	11	9	8	10	12	9	8	9
,, ,, MgO ,,	12	17	14	17	17	12	13	16	12	13	12	13	12	10	11	13	11	12	11	10
,, ,, CaO ,,	5	6	6	6	6	4	5	6	5	6	4	6	5	5	5	5	5	5	4	5
ROOTS																				
Dry weight (g.)	0·72	0·89	0·74	0·72	0·83	0·61	0·86	0·71	0·81	0·72	0·67	0·59	0·94	0·74	0·84	0·70	0·96	0·88	1·01	0·96
Absolute amount total N (mg.)	16	19	19	21	19	14	21	21	18	17	18	18	20	15	22	25	19	21	27	34
,, ,, K_2O ,,	16	18	21	30	16	16	27	31	21	24	25	20	30	26	38	39	40	44	57	59
,, ,, P_2O_5 ,,	5	7	6	8	7	5	8	8	6	6	6	6	7	6	7	8	7	7	9	11
,, ,, MgO ,,	8	11	7	7	10	6	8	6	9	7	6	5	9	6	7	6	9	8	8	7
,, ,, CaO ,,	3	4	3	4	4	3	3	4	5	4	3	3	4	4	4	4	4	4	4	5

966.54

not previously examined, the reverse is true, the K_2O content increasing rapidly with rising M.C.P.A. concentration.

The visual observations and mean plant dry weights indicate that the effect of M.C.P.A. on the tomato plant is not alleviated by an increase in the supply of potash to the roots, even though this additional potassium leads to a higher over-all potassium status within the tissues. The M.C.P.A. symptoms are clearly not due to a simple deficiency of potassium in the 'tops', since it may be seen from Table III that the K_2O content of the 'tops' of the plants receiving the highest M.C.P.A. treatment in conjunction with the highest potash supply is about equal to that of the controls of the lowest potash group.

From the data of Table V it is seen that the very marked effect of M.C.P.A. on the K_2O content of the 'tops' and roots is unique. Similar but very much less obvious trends, however, are shown by the P_2O_5 and total-N contents.

It is of interest to note that M.C.P.A. has no influence over the CaO content of any tissue and little if any effect on the MgO content. Similarly, visual assessment of the spectrographic plates could not detect any consistent differences in the Na, Mn, Ca, or Fe content of the tissues due to M.C.P.A. treatment. By contrast, the K_2O differences were readily apparent.

The changes in K_2O content of the tissues cannot be accounted for by the different dry weights between treatments, since there is little effect of M.C.P.A. during the period of the experiment on the dry weight of the roots, while the dry weight of the tops is markedly depressed. The following possibilities might account for the observed potash changes:

(*a*) An interference with the transport of potassium to the 'tops'.
(*b*) A redistribution of potassium from the 'tops' to the roots.
(*c*) A change in the rate of potassium uptake by the roots, superimposed on (*a*) or (*b*).

In the absence of data for the K_2O status of the plants at the beginning of M.C.P.A. treatment, it is not possible to decide between the first two possibilities.

Some information on the effect of M.C.P.A. on potassium uptake can, however, be obtained by a consideration of Table VII where mean values of the total N, K_2O, P_2O_5, MgO, and CaO contents over all potash levels, for the whole plants, have been calculated from the data of Table VI. It is thus seen that the K_2O content of the whole plant is markedly and progressively reduced with increasing M.C.P.A. concentration. This large depression is peculiar to potassium, no effect being seen for CaO and MgO, and only very slight depressions for P_2O_5 and total N. It seems likely, therefore, that M.C.P.A. brings about a specific inhibition of uptake of potassium. The reduction in potassium uptake by the roots may be due to the following:

(*a*) A real reduction of potassium uptake without any reduction in the absorbing surface of the roots.
(*b*) A reduction of active absorbing surface owing to morphological changes in the root system.

TABLE V

(*a*) *Mean Values of Dry Weights and Total N, K_2O, P_2O_5, MgO, and CaO Contents for increasing Potash Level over All M.C.P.A. Concentrations*

		Dry weight (g.)			Total N content (as % of dry wt.)			K_2O content (as % of dry wt.)			P_2O_5 content (as % of dry wt.)			MgO content (as % of dry wt.)			CaO content (as % of dry wt.)		
		Tops.	Lower stems.	Roots.	Tops.	Lower stems.	Roots.	Tops.	Lower stems	Roots.	Tops.	Lower stems.	Roots.	Tops	Lower stems.	Roots.	Tops.	Lower stems.	Roots.
Increasing potash	K(1)	1·38	0·89	0·77	3·85	1·71	2·54	4·7	3·2	2·8	1·34	0·94	0·84	1·9	1·7	1·0	1·2	0·6	0·5
	K(2)	1·47	0·94	0·75	3·86	1·59	2·49	5·2	4·1	3·0	1·29	0·94	0·89	1·9	1·4	1·0	1·4	0·6	0·5
	K(3)	1·62	0·90	0·70	3·73	1·54	2·60	6·3	5·2	3·7	1·30	0·91	0·88	1·7	1·4	1·0	1·2	0·6	0·5
	K(4)	1·67	0·98	0·80	3·73	1·50	2·58	6·8	6·5	4·3	1·25	0·95	0·88	1·8	1·2	0·9	1·2	0·5	0·5
	K(5)	1·84	1·07	0·95	3·61	1·50	2·67	7·5	7·4	5·3	1·17	0·91	0·91	1·6	1·0	0·8	1·1	0·5	0·5

(*b*) *Mean Values of Dry Weights and Total N, K_2O, P_2O_5, MgO, and CaO Contents for increasing M.C.P.A. Concentrations over All Potash Levels*

		Dry weight (g.)			Total N content (as % of dry wt.)			K_2O content (as % of dry wt.)			P_2O_5 content (as % of dry wt.)			MgO content (as % of dry wt.)			CaO content (as % of dry wt.)		
		Tops.	Lower stems.	Roots.	Tops	Lower stems.	Roots.	Tops.	Lower stems.	Roots.	Tops.	Lower stems.	Roots.	Tops.	Lower stems.	Roots.	Tops.	Lower stems.	Roots.
Increasing M.C.P.A.	(*a*)	2·06	0·94	0·86	3·90	1·69	2·22	7·2	6·3	2·8	1·39	1·02	0·78	1·6	1·3	1·1	1·2	0·6	0·5
	(*b*)	1·89	0·93	0·77	3·70	1·59	2·25	6·8	5·5	3·4	1·32	0·94	0·79	1·8	1·4	1·0	1·2	0·6	0·5
	(*c*)	1·51	0·90	0·82	3·71	1·44	2·61	5·9	4·9	4·1	1·24	0·86	0·86	1·8	1·4	0·9	1·3	0·6	0·5
	(*d*)	0·94	1·04	0·74	3·71	1·54	3·22	4·4	4·4	5·1	1·17	0·91	1·09	1·9	1·4	0·8	1·2	0·6	0·5

TABLE VI

(*a*) *Mean Values of Dry Weights and Absolute Amounts of Total N, K_2O, P_2O_5, MgO and CaO for increasing Potash Level over all M.C.P.A. Concentrations*

		Dry weight (g.)			Absolute amount of N (mg.)			Absolute amount of K_2O (mg.)			Absolute amount of P_2O_5 (mg.)			Absolute amount of MgO (mg.)			Absolute amount of CaO (mg.)		
		Tops.	Lower stems.	Roots.	Tops.	Lower stems.	Roots.	Tops.	Lower stems.	Roots.	Tops.	Lower stems.	Roots.	Tops.	Lower stems.	Roots.	Tops.	Lower stems.	Roots.
Increasing potash ↓	K(1)	1·38	0·89	0·77	52	15	19	66	27	21	19	8	6	27	15	8	17	6	4
	K(2)	1·47	0·94	0·75	56	15	19	79	38	23	19	9	7	28	13	7	20	6	4
	K(3)	1·62	0·90	0·70	60	14	18	105	46	25	21	8	6	28	12	7	19	5	4
	K(4)	1·67	0·98	0·80	62	15	21	119	63	33	21	9	7	28	12	7	20	5	4
	K(5)	1·84	1·07	0·95	66	16	25	143	78	50	22	10	9	29	11	8	21	5	4

(*b*) *Mean Values of Dry Weights and Absolute Amounts of Total N, K_2O, P_2O_5, MgO and CaO for increasing M.C.P.A. Concentrations over All Potash Levels*

		Dry weight (g.)			Absolute amount of N (mg.)			Absolute amount of K_2O (mg.)			Absolute amount of P_2O_5 (mg.)			Absolute amount of MgO (mg.)			Absolute amount of CaO (mg.)		
		Tops.	Lower stems.	Roots.	Tops.	Lower stems.	Roots.	Tops.	Lower stems.	Roots.	Tops.	Lower stems.	Roots.	Tops.	Lower stems.	Roots.	Tops.	Lower stems.	Roots.
Increasing M.C.P.A. ↓	(*a*)	2·06	0·94	0·86	79	16	18	152	61	25	29	10	7	33	12	9	24	5	4
	(*b*)	1·89	0·93	0·77	69	15	17	131	51	26	25	9	6	33	13	7	23	5	4
	(*c*)	1·51	0·90	0·82	56	13	21	90	44	34	19	8	7	28	12	7	19	5	4
	(*d*)	0·94	1·04	0·74	35	16	24	42	46	37	11	9	8	18	14	6	11	6	4

The former appears more likely since the effect seems to be confined to the potassium ion.

It is most improbable that the altered K_2O content of the tissues can be the result of changes in their histology alone, since from Table V (*b*) it may be seen that in the lower stem, where adventitious root initials are present, the K_2O content of the tissue is still greatly diminished as compared with the controls. However, the much smaller observed changes in total N and P_2O_5 contents of the various portions may conceivably be due to this cause.

TABLE VII

Mean Values of Dry Weights and Total N, K_2O, P_2O_5, MgO, and CaO Contents of Whole Plants expressed as Percentages of the Dry Weights for increasing M.C.P.A. Concentration over all Potash Levels

M.C.P.A. concentration.		Dry weights of whole plants (g.).	Total K_2O content (as % of dry wt.).	Total P_2O_5 content (as % of dry wt).	Total MgO content (as % of dry wt.).	Total CaO content (as % of dry wt.).	Total N content (as % of dry wt.).
Increasing ↓ M.C.P.A.	(*a*)	3·86	6·2	1·19	1·4	0·9	2·93
	(*b*)	3·59	5·8	1·11	1·5	0·9	2·81
	(*c*)	3·23	5·2	1·05	1·5	0·9	2·79
	(*d*)	2·72	4·6	1·03	1·4	0·8	2·76

It may be concluded, therefore, that M.C.P.A. applied to the roots of a susceptible species, tomato (variety Stonor's M.P.) in sub-lethal amounts, has a specific effect on the potassium metabolism of the plant, causing a marked diminution of the K_2O content of the 'tops' and lower stems and a marked accumulation of potassium in the roots, the K_2O content of the plant as a whole being greatly lowered. These effects appear to be due to a specific inhibition of uptake of potassium by the roots combined with either an interference with the transport of potassium from the roots to the tops or with a redistribution of potassium from the tops to the roots. Further investigation of these effects may help in determining the mode of action of M.C.P.A. and may also lead to a better understanding of the role of potassium in plant nutrition.

It is possible, therefore, that the effects of the sub-lethal concentrations of M.C.P.A. on the mineral nutrition of a susceptible species, described in this paper, may be observed in a more severe form in susceptible species sprayed under field conditions with the usual phytocidal amounts of M.C.P.A. and that they may contribute to the death of the plant. The rapidity with which these changes in K_2O content of the tissues under the influence of M.C.P.A. can occur and the manner in which they are brought about is now being investigated, together with their relationship to the changes in carbohydrate metabolism reported by Mitchell (1946) as being induced by a closely related compound, 2-4-dichlorophenoxyacetic acid.

APPENDIX

Methods of analysis. Owing to the intention to examine the tissues with regard to their content of a fairly wide range of elements and because of the small amounts of material available, it was decided that only total nitrogen and phosphate could be satisfactorily determined by chemical methods. Total nitrogen was determined by the usual semi-micro Kjeldahl technique using sodium sulphate and selenium during digestion and boric acid for ammonia absorption in the distillation. Phosphate was determined on a separate 0·05 g. of oven-dry material by digestion with sulphuric and nitric acids. The development of colour was carried out by a modification of the method reported by Greenhill and Pollard, measurement being made on a Hilger 'Spekker' absorptiometer using 1-cm. cells and No. 1 red glass filters. A standard curve was constructed, from which the 'unknowns' were calculated.

All other elements were determined by a spectrographic method of analysis. For K, Ca, and Mg, chemical determinations on selected samples were used for calibration of the spectrographic plates which were assessed photometrically. In the case of Na, Mn, Cu, and Fe, visual inspection of the plates showed that there were no large or consistent differences between the treatments, and therefore no photometric determinations were made.

Spectrographic analysis. A cathode layer arc method using lathe-turned carbon electrodes (5-mm. rod, 2·5 cm. long, turned to have a shank 1 cm. long of 3·0 mm. diameter bored 1·5 mm. to a depth of 6 mm.) and a current of 6 amps. The arc was focused on the partially masked colimator in the conventional style and a rotating sector was employed to cut down the total light. It was found that arcing at 6 amps. for 2½ minutes with the sector transmitting ¼ of the total light gave suitable images on the plates (Ilford Special Lantern, normal, contrast, developed 2 minutes at 18° C. in I.D.2).

No internal standard was used as no one element was considered suitable for K, Ca, and Mg at one and the same time. Photometric measurements were made of line strength and adjacent background. The lines used were

K	3446·4 Å.
Ca	3158·9 Å.
Mg	2776·7 Å.

For the routine spectrographic analysis a complete replicate of 'tops', lower stems, or roots was arced in duplicate in the carbon electrodes under identical conditions. After the plate had been processed, four samples forming a suitable concentration range were picked for chemical analysis for K, Ca, and Mg. These same samples were then used as standards for the construction of curves by the photometric measurement of line density.

The spectrographic plates served as permanent records and were later visually examined for differences in Na, Mn, Cu, and Fe.

Chemical determination of Ca, Mg, *and* K. Owing to the small quantities of material available for analysis, the usual macro-techniques were unsuitable. A micro-method modified from the procedures employed by Chapman and Eden was devised and found to give satisfactory results. The reagents used were as follows:

1. Alcoholic ammonium hydroxide: 5 ml. 0·88 ammonia solution diluted with 75 ml. alcohol and adjusted to 100 ml. with water.

2. Reducing agent: 0·5 g. amino naphthol sulphonic acid 1:2:4 added to 27 g. sodium bisulphite in 180 ml. water. 20 per cent. sodium sulphite was added to bring the volume to 200 ml. Filter.
3. Sodium cobaltinitrite: (*a*) 25 g. cobalt nitrate crystals dissolved in 50 ml. water to which 25 ml. acetic acid was added.
   (*b*) 150 g. sodium nitrite dissolved in 250 ml. water.
   (*a*) and (*b*) were mixed and air bubbled through for 24 hours to remove oxides of nitrogen. This was filtered.
4. Standard magnesium solution: 0·959 g. mono potassium phosphate was dissolved to 1 litre. This stock solution was diluted 10 times, when 1 ml. = 50 μg. P_2O_5 = 28 μg. MgO.

The following procedure was adopted:

0·0500 g. of oven-dry sample was ashed at 450° in Pyrex ignition tubes 4 × 1 cm. each provided with small side tube near its base. These were found far superior to small dishes for ashing, baking, and transference. It was convenient to have an asbestos rack upon which these tubes could be rested whilst ashing, &c. The ash was treated with a few drops of HNO_3 and evaporated to dryness on a hot plate, when ashing was repeated. The final residue after treatment with acid and baking was digested with a few drops of 2N HCl on a hot plate and filtration and washing was made into a 10-ml. centrifuge tube using suction. The extract was diluted to approximately 4 ml. and 0·2 g. NH_4Cl added, together with 0·5 ml. 10 per cent. oxalic acid, one drop acetic acid, and two drops bromo cresol green indicator. After standing in a beaker of boiling water for 5 minutes, dilute ammonia ('0·880' diluted 4 times) was added dropwise with stirring until the first shade of emerald green appeared. After a further ½ hour in the beaker, the tubes were cooled and centrifuged at 2,500 r.p.m. for 5 minutes and the supernatant liquid poured off into a 30-ml. beaker, together with four subsequent washings of 2 ml. of water. The residue was dissolved by heating in a water-bath with 1 ml. 10N H_2SO_4 plus a little water. Titration was carried out hot using N/50 $KMnO_4$, when 1 ml. N/50 $KMnO_4$ = 0·00056 g. CaO.

The solution in the 30-ml. beaker was transferred to a 25-ml. graduated flask and diluted to the mark. A 5-ml. aliquot was treated with 0·1 g. citric acid, 1 ml. '0·880' ammonia in a 10-ml. centrifuge tube, and then cooled in ice. To the cold solution 1 ml. of ice-cold 5 per cent. $(NH_4)_2HPO_4$ solution was added and stirred vigorously until precipitation occurred. After standing overnight at about 5° C., the precipitate was centrifuged out at 2,500 r.p.m. and washed three times with dilute ammonia and finally with alcoholic ammonia. 0·5 ml. of 10N H_2SO_4 was added, the precipitate dissolved by immersion for ½ hour in a water-bath. 6·5 ml. of water was added and any undissolved particles centrifuged to the bottom, 2 ml. 2·5 per cent. ammonium molybdate solution added, followed by 0·4 ml. reducing agent. After mixing by inversion and standing 15 minutes the colour was measured in a Hilger Spekker Absorptiometer using 1 cm. cells and No. 1 red glass filters. A standard curve was constructed from which the 'unknowns' were calculated.

A 10-ml. aliquot as above was transferred to a 30-ml. beaker and taken to dryness on a hot plate with a few drops of HNO_3. After ashing at 450° for 10 minutes, 2 ml. of water was added together with 1 drop of acetic acid and the whole warmed. The beaker was carefully 'policemaned' and then rinsed into a 10-ml. centrifuge tube, keeping the volume to approximately 4 ml, when 2·5 ml. of cobaltinitrite reagent was added with careful mixing and allowed to stand for 1 hour. Centrifuging was made at 2,500 r.p.m. for 5 minutes, followed by washing with 2 ml. of a saturated solution of $K_2NaCo(NO_2)_6$ and two lots of 2 ml. 70 per cent. alcohol. After draining, 2 ml. of water was added and solution caused by immersion in boiling water. 0·5 ml. 20 per cent. H_2SO_4 was added and the solution titrated hot with N/50 $KMnO_4$ when 1 ml. N/50 $KMnO_4$ = 157 μg. K_2O.

The potassium determinations made by this method were checked against the standard macro-method at frequent intervals. Since the work described here was completed, the modification proposed by Chapman (1947) has been tested and found more satisfactory.

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Multiple-allelomorph Incompatibility of Pollen and Style in the Evolution of the Angiosperms

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I. INTRODUCTION

THE sudden rise of the angiosperms in the Cretaceous period to a position of dominance in the Plant Kingdom, as suggested by fossil evidence, is one of the most remarkable features of plant evolution for which no satisfactory explanation has been offered. Considerable evolutionary significance has been attributed to the closed carpel as the outstanding character which distinguishes angiosperms from gymnosperms. It has been supposed that the closing of the investing carpel has given such added protection to the ovules as would account for the rise of the angiosperms, and only secondary importance has usually been attached to other evolutionary 'advances', such as the aggregation of the stamens and carpels into flowers which are often adapted for insect-pollination, extreme reduction of the male and female gametophytes, double fertilization, and the occurrence of vessels in the xylem. Protection of the ovules is usually considered to be either from desiccation or from injury by animals. However, there is no evidence to suggest that living gymnosperms are greatly handicapped by their lack of protection from these agents. Indeed in most conifers and cycads the ovules are only 'exposed' at the time of pollination, and are well protected by cone-scales at all other times, while in *Ginkgo*, *Podocarpus*, and *Taxus* the ovules are at all times freely exposed, showing that even moderate protection is not always essential for survival. It would thus appear that there is no single morphological character or combination of characters that will account satisfactorily for the remarkable evolutionary success of the angiosperms. Their outstanding

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character is adaptability: they show more diverse life-forms and have invaded more extreme habitats than any other group of vascular plants. Accordingly it is worth-while to consider the factors which may increase the power of adaptation of an organism to its environment.

Fisher (1930) and Muller (1932) have shown that the primary advantage of sexual over asexual reproduction lies in the recombination of hereditary differences, thus giving to sexual species the advantage of responding more rapidly than asexual species to the influence of natural selection and therefore an overwhelming advantage in the evolutionary race. Darlington (1939) and Mather (1940, 1943 *a*) have pointed out that outbreeding is essential for sexual reproduction to show this advantage. However, when a species has become well adapted to a particular environment, outbreeding devices may be discarded, since they are necessarily associated with the bringing together of nuclei from different individuals and this means there will always be a finite probability of sexual reproduction failing altogether. A self-fertile individual always has the advantage that sexual reproduction is independent of other individuals of the species. It is common to find that, within any family or genus of plants, some species possess outbreeding devices while others do not. It is probable that most self-fertile species have been derived from outbred ancestors, since their present adaptation could have been acquired most readily through cross-breeding. Mechanisms which reduce or prevent self-fertilization without greatly restricting the ease of cross-fertilization are therefore likely to have been of particular significance in the early stages of the evolution of a species or a group of species of common descent. The key to the success of the angiosperms may therefore be sought in devices which prevent self-fertilization and promote more efficient cross-fertilization than is possible in the gymnosperms, rather than in structural features which are often of less fundamental evolutionary significance.

II. COMPARISON OF OUTBREEDING DEVICES IN GYMNOSPERMS AND ANGIOSPERMS

There appear to be two main outbreeding devices in the gymnosperms, dichogamy and dioecism. By dichogamy, or the separation in the time of maturation of the pollen-sacs and ovules, cross-pollination is favoured but rarely made certain, since the times of maturation usually overlap. If they do not overlap, both cross- and self-pollination may be prevented. By dioecism, or the restriction of the pollen-sacs and ovules to different plants, self-pollination is made impossible, but at a heavy cost, since, if the sexes are distributed equally, any given individual can mate with only half the individuals in the population.

Dichogamy and dioecism also occur in the angiosperms: dichogamy here relates to the times of maturation of the pollen-sacs and stigmas. Numerous structural adaptations which favour cross-pollination occur in entomophilous flowers, but these do not prevent pollination occurring between different flowers on one plant, and so, in general, are merely 'aids' to cross-breeding.

Gynodioecious species, with female and hermaphrodite individuals in the population, occur in some families of angiosperms, but are less effectively outbred than dioecious species, since the hermaphrodites are self-fertile (see Mather, 1940; Lewis, 1942). Several other outbreeding devices are found in the angiosperms, based on incompatibility of pollen and style. These are worthy of special attention since no comparable mechanisms are known or indeed appear to be possible in gymnosperms.

Incompatibility is brought about by a physiological reaction between haploid pollen-tubes and diploid style, so that the growth of pollen-tubes of certain physiological constitutions is slowed down and eventually stopped, while others continue to grow normally. Two fundamentally different mechanisms for the genetic control, and hence the inheritance, of incompatibility are known in angiosperms (Mather, 1944). In both mechanisms the physiological constitution of the diploid style is determined by its own genotype, but the physiological constitution of the haploid pollen may be primarily determined either by its own genotype or by the physiological constitution, and hence ultimately the genotype, of the diploid anther in which it was formed. Mather refers to these two types as having haplo-diploid and diplo-diploid control of incompatibility, respectively. The haplo-diploid type appears to have as its primary genetic basis a series of mutiple allelomorphs at one locus, while the diplo-diploid type, which is usually although not necessarily associated with heterostyly, appears to be controlled either by one or by two gene loci, each with one dominant and one recessive allelomorph, and with one dominant gene epistatic to the other when there are two loci. With haplo-diploid control, the number of self-incompatible cross-compatible classes is limited only by the number of allelomorphs in the population at the incompatibility locus, whereas with diplo-diploid control, where there are normally only two allelomorphs per locus, the number of possible classes is determined by the number of loci. Not more than two loci have been recorded, and, accordingly, not more than three self-incompatible cross-compatible classes of individuals are known. The types of incompatibility described by Mather (1944) as having haplo-diploid and diplo-diploid control may be called multiple-allelomorph and two-allelomorph incompatibility, respectively. This nomenclature has the advantage of emphasizing the essential similarity, both in genetic basis and in function, of pollen and style incompatibility in the angiosperms and physiological heterothallism in the fungi, where two-allelomorphic and multiple-allelomorphic types can also be recognized (Whitehouse, 1949*b*).

Two-allelomorph incompatibility, when determined by one locus, gives rise to two self-incompatible cross-compatible classes of individuals in a species. When the essential physiological differentiation of the two classes is also associated with a morphological difference, as in distylic plants, then the two classes may be recognized by their short- and long-styled flowers, respectively. In *Armeria maritima* and many other species of Plumbaginaceae, a dimorphism of pollen, or of stigmas and pollen, is known, associated with an incompatibility analogous to that of distylic plants, but with no macroscopic difference between the two types of flowers (see Baker, 1948). This suggests that the essential feature of the incompatibility mechanism is the physiological and not the morphological differentiation of the two classes of individuals. With incompatibility of the two-allelomorphic type determined by one locus, self-fertilization is prevented, but, as with dioecism, only at the cost of reducing the number of possible mates for a given individual to approximately half the total number of individuals in the population. The system has an advantage over dioecism in that every individual is bisexual, but nevertheless the free transfer of genes through the population is severely restricted. It cannot therefore be supposed that the acquisition of incompatibility determined by two allelomorphs at one locus would give the angiosperms any marked superiority over the gymnosperms.

Two-allelomorph incompatibility determined by two loci gives rise to three self-incompatible cross-compatible classes of individuals. These are familiar in plants with tristylic flowers such as *Lythrum salicaria*, where the three classes of individuals are morphologically distinct. In such plants a physiological device is found which appears to elaborate the incompatibility mechanism without being an essential part of it. Each individual has flowers with anthers at two different levels. The pollen from these stamens is physiologically differentiated so that it is fertile only upon a style which has its stigmatic surface at the corresponding level. That this physiological selectivity associated with the morphological differentiation of the three classes of individuals is not essential for the action of the incompatibility mechanism is suggested by the discovery by Riley (1936) that *Capsella grandiflora* shows an incompatibility of pollen and style essentially similar to that of tristylic plants in its genetic basis, since the behaviour of the pollen is controlled, not by its own genotype, but by the influence of the diploid anther in which it was formed. In this species the three classes of individuals, though physiologically distinct, are morphologically alike.

When two-allelomorph incompatibility is determined by two loci, self-fertilization is prevented with less restriction on cross-fertilization than when it is determined by one locus, since with three classes of individuals, if all are equally frequent in the population (the optimum condition), any given individual can mate with two-thirds of the total number of individuals. Furthermore, unlike a dioecious species, every individual is bisexual and potentially seed-fertile. Two-allelomorph incompatibility with two loci thus provides a more efficient cross-fertilizing device than is known in the gymnosperms, but the gain in cross-breeding efficiency as compared with that of dioecious species is not great. Tristyly appears to be known in only four families of flowering plants—Pontederiaceae, Amaryllidaceae, Oxalidaceae, and Lythraceae. It is of course possible that two-allelomorph incompatibility determined by two loci and without morphological differentiation may be widespread in the angiosperms, but since such investigations as have been made of incompatibility in homostylic plants have revealed only one instance of it (*Capsella grandiflora*), it must be concluded that it is probably of relatively rare occurrence. It is therefore unlikely that the success of the angiosperms as a whole can be attributed to two-allelomorph incompatibility determined by two loci.

Multiple-allelomorph incompatibility is the commonest type of incompatibility found in homostylic flowering plants. As previously stated, it has as its genetic basis a multiple-allelomorphic series at one locus, such that pollen-tubes having an allelomorph in common with the tissue of the style have a reduced growth-rate and fail to reach the ovules. Thus, if the allelomorphs are denoted by S_1, S_2, S_3, &c., then the growth of S_1 and S_2 pollen-tubes is inhibited on an S_1 S_2 style, but that of S_3, S_4, S_5, &c., pollen-tubes is not. The number of allelomorphs at the incompatibility locus in wild or cultivated populations has been found to be large in every species that has been investigated. Thus, East and Yarnell (1929) found 15 different allelomorphs in a sample of 32 allelomorphs (16 plants) of *Nicotiana alata grandiflora* and hybrids of this species with *N. Forgetiana*. Other experimental data by various authors are quoted by Williams (1947). One of the most thorough investigations has been that of Emerson (1940), who found 45 allelomorphs in *Oenothera organensis*, where the total natural population of the species consists of only about 500 individuals. Extensive studies have been made on natural and cultivated populations of species of *Trifolium*, and in every instance a large number of allelomorphs has been found. The published data are presented in Table I. In the last column the estimated total number of allelomorphs in each population is given, based on the assumption that all the allelomorphs are equally frequent in the population. The method given by Whitehouse

TABLE I

Data from Experimental Investigations of the Number of Incompatibility Allelomorphs in Populations of Species of Trifolium

Species.	Author and reference.	Source of plants studied.	No of individuals in which both alleles were identified.	No of individuals in which one allele was identified.	Maximum no. of alleles possible in sample.	No. of different alleles recorded.	Estimated total number of alleles in population.
Trifolium pratense	Williams, W. (1947), from data of Williams, R. D. (1939)	Non-pedigree population of 'English late-flowering red clover'.	24	0	48	41	150
		Non-pedigree population of 'English broad red clover'.	18	2	38	34	170
Trifolium repens	Atwood (1942)	Widely separated localities in America, and one plant from New Zealand.	9	1	19	18	175
		Widely separated localities in America.	17	0	34	28	85
	Atwood (1944)	Isolated natural pasture of approximately 1 acre.	0	49	49	36	75
		Natural pasture of nearly 100 acres.	0	49	49	39	100
Trifolium hybridum	Williams, W. (1948)	Canadian commercial seed population.	10	1	21	13	20

(1949*a*) for estimating the number of allelomorphs at the loci for heterothallism in fungi is equally applicable to incompatibility in flowering plants and has been used to obtain these figures. The method of Bateman (1947) gives approximately the same figures. It is evident that the number of allelomorphs at the incompatibility locus in populations of species of *Trifolium* is usually of the order of magnitude of 100. This means that the cross-fertilizing efficiency of these plants will be very near to 100 per cent. In fact, Atwood (1944) has found that there was only about 1 per cent. of cross-incompatibility in natural populations of *Trifolium repens*.

This extraordinarily efficient device for preventing self-fertilization, and yet hardly restricting cross-fertilization, is already known to have a very widespread distribution in the angiosperms. East (1940) records the known distribution of incompatibility in flowering plants, but he does not discriminate between the two-allelomorphic and multiple-allelomorphic types. However, in view of the apparent rarity of two-allelomorph incompatibility in homostylic plants, it appears justifiable to conclude that the great majority of instances of incompatibility that are not associated with any morphological differences between the cross-compatible individuals will prove to be of the multiple-allelomorphic type. East (1940) records the occurrence of incompatibility not associated with heterostyly in over 150 genera of angiosperms distributed through 38 families[1] and 19 orders, and including both Monocotyledons and Dicotyledons. In addition, incompatibility has been reported in *Corylus* (Betulaceae) by Schuster (1924). Since only a very small proportion of the total number of species of angiosperms has been tested experimentally for incompatibility, the true distribution is undoubtedly much wider than the existing data have revealed.

East (1940) has pointed out that incompatibility appears to have played an important part in the evolution of the flowering plants, but he does not attempt to estimate the relative evolutionary importance of the two main types of incompatibility. Mather (1940) considers that the primary advantage of incompatibility over dioecism as an outbreeding device lies in the reduction of gametic loss, since in many incompatible species there are fewer individuals upon which pollen from a given flower fails to function. In a later paper (Mather, 1944) he compares the efficiency of the two main types of incompatibility, and points out that the cross-breeding efficiency is higher and the gametic loss lower with haplo-diploid (multiple-allelomorphic) incompatibility than with the diplo-diploid (two-allelomorphic) type. He also points out that whereas multiple-allelomorph incompatibility requires a minimum of

[1] Gramineae, Bromeliaceae, Commelinaceae, Liliaceae, (Amaryllidaceae), Iridaceae, Zingiberaceae, Orchidaceae, Nyctaginaceae, Nymphaeaceae, Ranunculaceae, Papaveraceae, Cruciferae, Resedaceae, Saxifragaceae, Rosaceae, Leguminosae, Geraniaceae, Euphorbiaceae, Malvaceae, Passifloraceae, Begoniaceae, (Lythraceae), Onagraceae, (Primulaceae), (Plumbaginaceae), Apocynaceae, Asclepiadaceae, (Polemoniaceae), (Boraginaceae), Solanaceae, Scrophulariaceae, Acanthaceae, (Rubiaceae), Caprifoliaceae, Campanulaceae, Goodeniaceae, Compositae. Families enclosed in brackets contain heterostylic species (two-allelomorph incompatibility), and hence incompatibility recorded in the homostylic species of these families is more likely to be of the two-allelomorphic type than it is in the other families.

three allelomorphs for its operation, the two-allelomorphic type requires only two, and consequently the latter type could probably arise in evolution more readily than the former. He concludes that the relative evolutionary advantages of the two types of incompatibility do not differ markedly, the lower cross-breeding efficiency and greater gametic loss of the two-allelomorphic type being compensated by the greater likelihood of its originating by mutation.

The significance of cross-breeding efficiency appears to have been greatly underestimated in these evaluations of the relative importance in evolution of dioecism, two-allelomorph and multiple-allelomorph incompatibility. Since all three of these mechanisms entirely prevent self-fertilization, their outbreeding efficiencies have been regarded as maximal and thus equal. Indeed if outbreeding is measured solely by the degree of heterozygosis in the individuals of the population, then clearly all three methods achieve heterozygosis for every individual, and, if mating is spatially at random, all methods will be equally efficient. However, the cost of achieving such prevention of inbreeding should be measured by the extent to which cross-breeding is also restricted. With dioecism and two-allelomorph incompatibility this cost is heavy, but with multiple-allelomorph incompatibility, if the number of allelomorphs is large, cross-fertilization is almost unrestricted. Consequently, the chance of fertile cross-pollination occurring and of new favourable gene combinations appearing is greater in species with multiple-allelomorph incompatibility than in those with dioecism or two-allelomorph incompatibility. Fisher (1930) has shown that natural selection has little influence in promoting the spread of a favourable mutant gene when that mutation first appears. Thus any other mechanism which favours wide and rapid dispersal of genes through the population of a species will reduce the risk of mutant genes being lost, since so long as a gene is confined to a comparatively few individuals, there is a fairly high probability that these may ultimately leave no descendants. Hence, in general, a favourable mutation will have to appear fewer times in a multiple-allelomorph incompatible species than in a dioecious or two-allelomorph incompatible species, before it is likely to establish itself and spread through the population. Thus, given suitable environmental conditions, species possessing multiple-allelomorph incompatibility will be capable of a higher rate of evolution and have a greater power of adaptation than species without it. Multiple-allelomorph incompatibility therefore appears to be of much greater evolutionary significance than either dioecism or two-allelomorph incompatibility, and may indeed be the key to the success of the angiosperms.

It is significant, moreover, that the analogous phenomenon of multiple-allelomorph heterothallism in the fungi appears to be characteristic of the Hymenomycetes and Gasteromycetes, where it is evidently of extremely widespread occurrence, and may well be the primary cause of the evolutionary success of these Higher Basidiomycetes (Whitehouse, 1949*a*, *b*). A comparison of the efficiency of the main genetic systems known in the Plant Kingdom for restricting or preventing inbreeding, while maintaining a considerable degree of cross-fertility, is shown in Table II. It will be observed that the outbreeding systems known in the fungi find an exact parallel in those known in the angiosperms. However, the outbreeding devices of plants such as the angiosperms with sexual differentiation determined by the diploid phase are more efficient than their counterparts in such plants as the fungi with no sexual differentiation of the diploid phase, since in the latter, unlike the former, self-fertilization of haploids derived from one diploid is never entirely prevented (see Mather, 1942). It is evident from Table II that multiple-allelomorph incompatibility in the angiosperms is an almost perfect outbreeding device, superior to that known in any other group of plants.

TABLE II

Comparison of the Efficiency of the Main Genetic Systems known in the Plant Kingdom for reducing Self-fertility while maintaining Cross-fertility. In the Column headed Self-fertility is given the Mean Percentage Frequency of Fertile Matings between two Haploid Individuals (in A) or two Gametes (in B) chosen at Random from the Progeny of any one Diploid, Dikaryon, or Fertile Haploid-pair. Under Cross-fertility is given the Mean Percentage Frequency of Fertile Matings between Individuals (Haploid in A, Diploid in B) chosen at Random from the General Population of the Species

Outbreeding system.	Genetic basis where known.	Known distribution.	Self-fertility.	Cross-fertility.
	A. *Plants with no sexual differentiation in diploid phase.*			
Haplo-dioecism	Pair of differential chromosome segments (one sex with X, the other with Y).	Algae; Fungi; Bryophyta.	50 %	50 %
Two-allelomorph heterothallism.	Pair of allelomorphs at one locus.	Algae; Fungi.	50 %	50 %
Multiple-allelomorph heterothallism.	Multiple allelomorphs at one or two loci.	Fungi (Hymenomycetes and Gasteromycetes).	50 % (one locus) or 25 % (two loci)	Almost 100 %
	B. *Plants with sexual differentiation determined by diploid phase.*			
Dioecism.	Pair of differential chromosome segments (one sex XX, the other XY).	Algae (*Fucus*, *Codium*); Gymnosperms; Angiosperms.	0 %	50 %
Two-allelomorph incompatibility.	One or two loci each with a dominant and a recessive allelomorph; when two loci, one dominant gene epistatic to the other.	Angiosperms (Heterostylic species; many Plumbaginaceae; *Capsella grandiflora*).	0 %	50 % (one locus) or 66·7 % (two loci)
Multiple-allelomorph incompatibility.	Multiple allelomorphs at one locus.	Angiosperms.	0 %	Almost 100 %

Accordingly, in the next two sections of this paper an outline will be given of the probable evolutionary consequences of the acquisition by a gymnospermous plant with well-protected ovules, of multiple-allelomorph incompatibility between the pollen and the tissue protecting the ovules. The course of evolution that would be expected to follow is compared with the known facts of the evolution of the angiosperms.

III. PROBABLE EVOLUTIONARY CONSEQUENCES OF THE ACQUISITION BY A GYMNOSPERMOUS PLANT WITH WELL-PROTECTED OVULES OF MULTIPLE-ALLELOMORPH INCOMPATIBILITY BETWEEN THE POLLEN AND THE PROTECTING TISSUE

The available evidence suggests that closure of the carpel was not an easy step in the evolution of the flowering plants, and that it conferred little immediate advantage upon those species which acquired it. Indeed, the germination of pollen-grains upon the 'stigma' of a closed carpel would appear to present greater hazards than germination upon the nucellus of individual ovules, in that the pollen-tubes would be faced with a longer and thus more precarious journey to the embryo-sacs. However, in a plant with well-protected ovules, that is, with a nearly closed carpel, if pollen sometimes germinated upon the carpel tissue instead of at the micropyles of the ovules, and if the pollen-tubes occasionally penetrated the carpel tissue successfully and reached the ovules, then a physiological antagonism developing between the cells of the carpel and pollen-tubes of similar genetic constitution could become highly effective in promoting cross-fertilization. Nevertheless, it appears unlikely that such an antagonism could attain sufficient selective value in a gymnosperm, since the thickness of nucellar tissue which the pollen-tubes must penetrate to reach the embryo-sac is hardly sufficient to be capable of interposing a physiological barrier. In the cycads the nucellus is comparatively thick, but since the pollen-tubes retain their original function of absorption of nourishment and do not act as sperm-carriers, little opportunity would appear to be offered for the operation of an incompatibility mechanism based on the inhibition or retarding of growth of pollen-tubes. Mather (1944) has indicated that incompatibility controlled by multiple allelomorphs is not likely to evolve easily, since a minimum of three allelomorphs is required before the system can begin to operate. However, as soon as an antagonism appears between carpel tissue and pollen tubes of similar constitution, the ovules of a monoecious species would be protected from the individual's own pollen, and circumstances would be favourable for the subsequent development of the highly efficient cross-breeding device of multiple-allelomorph incompatibility.

Such development would depend upon (*a*) the chance occurrence of the necessary three initial allelomorphs at the incompatibility locus, and (*b*) such conditions as a changing environment, under which natural selection would favour the occurrence of the maximum degree of outbreeding with its associated high frequency of recombination. Natural selection would then favour the development of a large number of allelomorphs at the incompatibility locus. It would also favour complete closing of the carpel, thus eliminating the risk of some pollen escaping the incompatibility 'sieve'. Furthermore, natural selection would favour the development and maintenance of a considerable thickness of carpel tissue through which the pollen-tubes must grow. This would afford opportunity for expression of the differential growth-rate between compatibile and incompatible pollen-tubes, which forms the basis of the incompatibility mechanism.

The multiple-allelomorph incompatibility system, when first established, would almost certainly be confined to a single species, since the very improbability of its appearing at all renders highly unlikely the chance of its appearing and becoming established more than once in a short interval of time. This species would have a more efficient outbreeding mechanism than any contemporary species of vascular plants, and would thus be expected to show greater hereditary variability and hence greater plasticity and potential adaptability than other species. In a suitable environment it would be expected to succeed and spread, and through the normal action of isolating mechanisms to become split into distinct species, which in their turn might also evolve new forms and undergo speciation. Such new species would likewise be expected to show greater hereditary variability and hence greater adaptability than their gymnospermous ancestors, both on account of their highly efficient outbreeding mechanism, and also, as Fisher (1930) has shown, because mutant genes can become established more readily in an increasing population than in a fixed one. Adaptability would render these species potentially successful in evolution, and would, in general, favour displacement of many gymnosperms by angiospermous plants. Under such changing conditions, with the dominant plants of the earth being replaced by new species, conditions would be ideal for an efficient outbreeding mechanism to be favoured by natural selection. Conditions would thus be unstable: the greater the success achieved by the angiosperms the greater would be the opportunity of further success.

Thus it seems likely that the first angiosperm with multiple-allelomorph incompatibility might soon give rise to a rapidly diverging and multiplying, and highly successful group of plants. In the evolutionary race the gymnosperm would to a large extent be unable to compete with the angiosperm with multiple-allelomorph incompatibility owing to the greater efficiency of spreading and recombination of genes in the latter, in the same way that asexual organisms would be unable to compete with sexual organisms under changing environmental conditions requiring ready powers of adaptation.

Ultimately the environmental conditions for the individual angiosperm species might be expected to become more stable when the less adaptable species of gymnosperms had been eliminated and the supremacy of the angiosperms had been attained. This relative stabilization of the biotic environment would be expected to slow down the rate of evolution of new forms, for the following reasons: Firstly, the highly efficient outbreeding mechanism would no longer be of such significance, and the disadvantage necessarily associated with any outbreeding device, namely, the risk of fertilization failing altogether, would be likely to cause natural selection to favour the discarding of the incompatibility in many species. Mather (1943 *b*) has pointed out that a multiple-allelomorph incompatibility system can be counteracted, either by the development of a fertility allelomorph at the incompatibility locus, or by the action of modifying genes at other loci. The inbreeding that is likely to follow the development of self-compatibility would be expected to slow down evolutionary change in these species. Secondly, in all species, including those in which multiple-allelomorph incompatibility was retained, the more static environmental conditions would also tend to retard the rate of evolutionary change, since many of the species would have reached an optimum state of adaptation to their particular environment.

It would therefore be expected that, when the angiosperms had become dominant and comparative stability was reached, a marked retardation of the rate of angiosperm evolution might occur. In self-compatible species, if environmental conditions subsequently again favoured outbreeding, the development of such mechanisms as dichogamy, dioecism, and two-allelomorph incompatibility would be more likely than the reappearance of multiple-allelomorph incompatibility, owing to the unlikelihood of the chance appearance by mutation of the necessary minimum of three allelomorphs for the latter mechanism to begin to operate. Those species which retained multiple-allelomorph incompatibility would probably become the chief progenitors of new forms, while those species with less efficient outbreeding devices would be expected to evolve only slowly, and those which were often inbred would be expected to remain relatively unchanged.

IV. EVIDENCE FROM PRESENT KNOWLEDGE OF ANGIOSPERM EVOLUTION

The hypothetical course of evolution traced in the previous section shows obvious similarities to the known facts of angiosperm evolution. The following points of resemblance may be noted:

i. A very long interval of time, of the order of magnitude of 200 million years, appears to have elapsed between the rise of the gymnosperms to a position of abundance, which apparently occurred in Devonian or early Carboniferous times, and the rise of the angiosperms apparently in the mid-Cretaceous period. This long interval of time would be explained if, following the adaptation of the pollen-tube as a sperm-carrier, simple closure of the carpel and germination of the pollen upon a stigma was insufficient alone to give angiospermy a selective advantage in evolution, but required, in addition, the chance occurrence of multiple-allelomorph incompatibility.

ii. All known fossils of angiospermous plants appear to be related directly to living angiosperms. This fact supports the view that although a nearly closed ovary may have arisen in several different groups of gymnosperms, a fully closed ovary appears to have been acquired successfully only once.

iii. There appear to be very few living 'angiosperms' in which the carpel is not completely closed, and where such carpels occur, as in *Reseda*, it is likely that the condition is derived and not ancestral. Such semi-angiosperms might have been expected to occur frequently by analogy with the gradations found in gymnosperms between freely exposed ovules as in *Ginkgo*, *Podocarpus*, and *Taxus*, and well-protected ovules as in *Pinus* and most cycads. The rarity of the semi-angiospermous condition and its apparently derived character would be accounted for if, in the early stages of evolution of the angiosperms, selection had favoured complete closure of the carpel, as the present hypothesis presumes, with the result that no semi-angiospermous species survived.

iv. The following facts support the hypothesis that all living angiosperms have been derived from a single ancestral species: (*a*) the uniformity of the male and female gametophytes of the angiosperms; (*b*) the very widespread occurrence of double fertilization within the group; and (*c*) the uniform basic plan of the stamens, comprising two anther-lobes each with two pollen-sacs having a sub-epidermal thickened layer and a common line of dehiscence.

v. The hypothesis that a very rapid evolutionary divergence of the angiosperms occurred in the earliest phase of their existence is supported by the simultaneous appearance in the earliest angiosperm fossils of many diverse types.

vi. The hypothesis that the angiosperms rose abruptly to world dominance is supported by the fact that the earliest plant remains showing undoubted relationship to living angiosperms are a few isolated fossils from the Jurassic period, yet by the Upper Cretaceous period the angiosperms had become dominant on the earth.

vii. Many Cretaceous angiosperm fossils and most of those from the Eocene and later periods can be clearly related to living families of angiosperms (Thomas, 1936, 1947). This is in keeping with the hypothesis that in the later phase of their existence the rate of evolution within the angiosperms has in general been slow.

In addition to the above points of resemblance between the hypothetical course of evolution traced in the previous section and the known facts of angiosperm evolution, certain deductions, which may lie open to confirmation by further observation, can be drawn from the hypothesis.

Firstly, if the hypothesis is valid, it is probable that in the evolution of the various types of ovary, selection would favour the development and maintenance of a considerable thickness of carpel tissue through which the pollen-tubes must grow. Hence a long style would tend to be favoured, perhaps of basal origin, or associated with an inferior ovary. It is also possible that the transmitting tissue (see Thomas, 1934), along which the pollen-tubes pass from stigma to ovule, has evolved in relation to efficient functioning of the incompatibility mechanism. Furthermore, many features of the carpel structure of flowers, such as the so-called 'false-septum' of the Cruciferae and the superposition of the stigmas and ovary partitions in the Papaveraceae, may have evolved in response to the selective advantage of an increased thickness of carpel tissue interposed between stigma and ovule.

Secondly, the frequency and distribution of the occurrence of various outbreeding devices in gymnosperms and angiosperms should provide evidence bearing upon the truth of the hypothesis. This is discussed in the following section.

V. EVIDENCE FROM THE FREQUENCY AND DISTRIBUTION OF THE CHIEF OUTBREEDING DEVICES IN GYMNOSPERMS AND ANGIOSPERMS

The outbreeding devices which will be considered are dioecism, which is found in both gymnosperms and angiosperms, and two-allelomorph and multiple-allelomorph incompatibility, which are known only in angiosperms.

i. *Dioecism*

Among living gymnosperms, which comprise about 600 species, hermaphrodite flowers are unknown except as an abnormality, and all the species can be classified either as monoecious[1] or dioecious. From published data it would appear that approximately 50 per cent. of the species are dioecious, distributed as follows: All the 80 species of Cycadales and the one species of Ginkgoales are dioecious. About one-third of the 450 species of Coniferales appear to be dioecious, including most of the species of *Agathis*, *Araucaria*, *Cephalotaxus*, *Dacrydium*, *Juniperus*, *Podocarpus*, and *Taxus*. Almost all the 70 species of Gnetales appear to be dioecious.

Among fossil gymnosperms, hermaphrodite flowers are known only from certain genera of Bennettitales (Cycadeoidales), such as *Williamsoniella* and *Cycadeoidea*. It may be that the risk of inbreeding was reduced in these plants by dichogamy, perhaps associated with synchronization of the time of flowering of all the flowers on one plant. Throughout all the other groups of fossil gymnosperms, unisexual flowers seem to have prevailed, but whether individual species were monoecious or dioecious it is impossible to say.

In the angiosperms, with some 133,000 species, data concerning the relative frequencies of hermaphrodite, monoecious, and dioecious species are far from complete, but nevertheless are sufficient to show that the proportion of hermaphrodite species is quite different from that existing in the gymnosperms. Thus, Yampolsky, C. and H. (1925), on the basis of available data, found that 70 per cent. of the genera of angiosperms contain only hermaphrodite species, and only 5 per cent. of the remaining genera contain only dioecious species. In the British flora, according to the data of Lewis (1942), the frequencies of hermaphrodite, monoecious, and dioecious species are 92·2, 5·4, and 2·4 per cent. respectively. From information given by Cheeseman (1925), the corresponding figures for the flora of New Zealand appear to be approximately 77·5 per cent. hermaphrodite, 9 per cent. monoecious, and 13·5 per cent. dioecious. Although complete data are not available, there can be no doubt that a majority of the species of angiosperms have hermaphrodite flowers.

One of the outstanding differences between the reproductive structures of gymnosperms and angiosperms is thus the high frequency of occurrence of hermaphrodite flowers in the angiosperms and the rarity of this condition in the gymnosperms. This suggests that the evolution of the hermaphrodite flower of angiosperms was favoured by the occurrence of incompatibility of pollen and style, since, as already indicated, incompatibility appears to be unknown in the gymnosperms. Without incompatibility, species with hermaphrodite flowers are likely in general to be readily self-fertilized, since even when dichogamous the times of maturation of stamens and stigmas may overlap. Moreover, this and other devices which tend to preclude the self-pollination of individual flowers rarely prevent pollination between different flowers on the same plant. It appears from East's (1940) data on the occurrence of incompatibility in angiosperms that hermaphrodite flowers occur in almost all the species in which incompatibility of pollen and style has been demonstrated. This is in keeping with the view that the hermaphrodite flower typical of the angiosperms may have evolved successfully in consequence of the occurrence of incompatibility of pollen and style.

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[1] The term monoecious as used in this paper indicates species in which both sexes are borne on the same plant, but in which the individual flowers are unisexual.

Lewis (1942) has presented evidence for the view that dioecious species of angiosperms have in general been derived from monoecious or hermaphrodite ancestors. This evidence is obtained from the frequency and distribution of dioecism in the group and from genetical studies, and may be summarized as follows:

(*a*) *Systematic evidence.* Dioecism, though comparatively uncommon in angiosperms, is very widely distributed and frequently occurs in isolated species or genera of otherwise monoecious or hermaphrodite families.

(*b*) *Genetical evidence.* Darlington (1939) has shown that a series of stages can be traced in the degree of elaboration of the sex-determining mechanism in dioecious organisms. These steps are the following: (1) Unisexual individuals originate from bisexual by the action of one or two genes. (2) A structural chromosomal change occurs in the region of the sex chromosome which carries the sex genes, such that crossing-over is suppressed within this region. Differentiation of the sexes can then proceed, since genes determining secondary sexual characters remain permanently linked to the sex appropriate to them. One sex then has two X-chromosomes and the other one X- and one Y-chromosome. (3) Since the Y-chromosome never pairs with the Y-chromosome of other individuals of the species, the differential segment of this chromosome never undergoes crossing-over, and recombination of genes within this segment is virtually impossible. This is disadvantageous to the species, and in time the genes of the Y-chromosome are replaced by genes on the other chromosomes (autosomes), the Y-chromosome becoming inert. Sex determination is then dependent on the proportion of X-chromosomes to autosomes.

The majority of dioecious angiosperms that have been investigated possess a sex-determining mechanism which is at a comparatively early stage in this process of elaboration. This suggests that dioecism is of comparatively recent origin in these species (Lewis, 1942).

The conclusions, (*a*) that the evolution of the hermaphrodite flower of the angiosperms may be related to the occurrence of incompatibility, and (*b*) that dioecious angiosperms are mostly of comparatively recent evolutionary origin from monoecious or hermaphrodite types, support the present hypothesis.

ii. *Two-allelomorph incompatibility*

Apart from its occurrence in *Armeria maritima* and other members of the Plumbaginaceae with dimorphic pollen or stigmas, and in *Capsella grandiflora*, the known distribution of two-allelomorph incompatibility is represented by the distribution of heterostyly. East (1940) records the existence of heterostyly in 17 families[1] of angiosperms distributed through 11 orders, with a possibility of its occurrence in 3 other families.[2] In many of these families the heterostylic species are confined to a few genera, or even to part of one genus, as in *Linum*. Lewis (1943 *a*, *b*, 1944) has shown that the physiological mechanism of incompatibility in *Linum grandiflorum* is based on differences of osmotic pressure, whereas in *Primula obconica* and *P. sinensis* it is analogous to the mechanism of multiple-allelomorph incompatibility and probably dependent on a specific reaction between protein molecules. The scattered distribution of heterostyly and the variable physiological mechanism of the incompatibility associated with it, suggest that this type of incompatibility has evolved comparatively recently and independently in a number of widely separated families. This is in keeping with the present hypothesis. In the Rubiaceae, where a large number of genera show heterostyly, and in the Plumbaginaceae (see Baker, 1948), it appears likely that two-allelomorph incompatibility evolved comparatively early in the evolutionary history of each family.

iii. *Multiple-allelomorph incompatibility*

Data are at present inadequate for any generalization to be made in relation to the distribution of multiple-allelomorph incompatibility in the angiosperms. As already indicated, there are many species in which it is not known whether incompatibility is of the two-allelomorphic or multiple-allelomorphic type, though it is probable that multiple-allelomorph incompatibility occurs in the majority, on account of the much greater frequency of this type among the homostylic angiosperms which have been investigated. East (1940) records the highest frequency of occurrence of incompatibility not associated with heterostyly in the families Gramineae, Liliaceae, Papaveraceae, Cruciferae, Rosaceae, Solanaceae, Scrophulariaceae, and Compositae. In these families there are indications that probably 10–25 per cent. of the species show incompatibility. However, little significance can be attached to this list, since in a great many other families few or none of the species have been investigated. It cannot yet be stated categorically that there is no family in which multiple-allelomorph incompatibility does not occur, since all the species of one family have

[1] Pontederiaceae, Amaryllidaceae, Polygonaceae, Oxalidaceae, Linaceae, Erythroxylaceae, Hypericineae, Dipterocarpaceae, Turneraceae, Lythraceae, Primulaceae, Plumbaginaceae, Oleaceae, Gentianaceae, Polemoniaceae, Boraginaceae, Rubiaceae.

[2] Amarantaceae, Thymelaeaceae, Verbenaceae.

not yet been tested. However, it is probable that multiple-allelomorph incompatibility is absent in families composed entirely of dioecious species, since dioecism and incompatibility appear to have the same function of promoting outbreeding.

The fact that the frequency of occurrence of multiple-allelomorph incompatibility is comparatively low in living angiosperms is not at variance with the hypothesis that all angiosperms are descended from a species with this type of incompatibility, for the following reasons:

i. All efficient cross-fertilizing devices are automatically associated with a disadvantage in that there is a risk of failure to set seed, through lack of fertile pollination. Hence under stable environmental conditions the occurrence of a considerable proportion of self-fertile species would be expected (see also Mather, 1940).
ii. It appears that multiple-allelomorph incompatibility can be lost in evolution much more readily than it can be acquired, since, as already stated, it can be counteracted either by a self-fertility allelomorph or by modifying factors at other loci (see Mather, 1943 *b*).
iii. A considerable proportion of living species of angiosperms appear to be polyploid, and it appears that the multiple-allelomorph incompatibility mechanism frequently breaks down in polyploids (see Lewis and Modlibowska, 1942; Lewis, 1944).

It might be argued that the wide distribution of multiple-allelomorph incompatibility among the angiosperms implies an origin analogous to that already attributed to dioecism, namely a comparatively recent and independent development in a large number of different families. However, dioecism can originate by the chance appearance of two mutations in different individuals, one suppressing the male and the other the female organs (Lewis, 1942), while the evolution of multiple-allelomorph incompatibility is likely to occur extremely rarely, since, as already stated, it requires the chance appearance of at least three allelomorphs controlling pollen-tube growth before it can begin to operate (Mather, 1944).

From his survey of the distribution of incompatibility in the angiosperms, East (1940) has concluded that incompatibility is more frequent in herbaceous than in woody families and genera, even allowing for the fact that a larger proportion of herbaceous than woody species has been investigated experimentally. He argued from this that as the woody types were generally thought to be the older in evolution, incompatibility had evidently arisen comparatively recently in many different lines of herbaceous plants. However, if the original angiosperms had all possessed multiple-allelomorph incompatibility, those species which subsequently lost it would be expected to evolve more slowly and hence to show a greater range of ancestral characters than those species which retained it. Thus the woody character, if ancestral, may have remained in those species which lost their incompatibility mechanism, and conversely, the herbaceous 'modern' families may be of comparatively recent origin because they have retained this mechanism. East's findings are therefore not necessarily at variance with the present hypothesis.

The idea that multiple-allelomorph incompatible species occupy the vanguard of evolution in the angiosperms, followed by a trail of less efficiently outbred species evolving more slowly, and with inbred species almost static in the rear, can of course only be applied broadly, since in individual instances many other factors will interact to modify the rate of evolution. It does suggest, however, that when the data concerning the distribution of multiple-allelomorph incompatibility in the angiosperms are more complete, they may provide evidence of the trend of evolution of different groups within the angiosperms.

When sexual reproduction first evolved, it must have caused a complete transformation of the dominant living organisms of the earth, owing to the inability of asexual species to compete with sexual species. All subsequent abrupt increases in the efficiency of sexual reproduction as a means for recombining hereditary differences are likely to have been followed by similar widespread changes, though probably on a smaller scale than that which must have followed the evolution of sexual reproduction itself. The evidence presented in this paper supports the conclusion that the abrupt rise of the angiosperms from the gymnosperms may have been the result of such a transformation, due to the increased efficiency of sexual reproduction made possible by multiple-allelomorph incompatibility.

VI. SUMMARY

It is suggested that multiple-allelomorph incompatibility of pollen and carpel tissue has been the primary cause of the evolution of the closed carpel and of the success of the angiosperms over their gymnospermous ancestors. The profound significance of the closed carpel for angiosperm evolution would then lie in the protection of the ovules, not from desiccation or the attacks of animals, but from fertilization by the individual's own pollen, without appreciably restricting cross-fertilization. This hypothesis is supported by the wide distribution of multiple-allelomorph incompatibility in living angiosperms, and it appears to account for many of the known facts of angiosperm evolution and for their outstanding quality, adaptability.

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The Symbiotic Seed-borne Fungus in the Cistaceae

I. Distribution and Function of the Fungus in the Seeding and in the Tissues of the Mature Plant[1]

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With Plate VII and six Figures in the Text

INTRODUCTION

INVESTIGATIONS on the structure and life-history of those fungi which are found in the testa or in the seed tissues of the angiosperms, and the effect of their presence on the seed and its consequent germination, have been carried out by numerous workers. Parasitic fungi frequently contaminate seeds, and the danger in infection of this kind is the carrying over of the fungus from the seed to the next generation (Orton, 1924, 1931; Doyer, 1948).

The majority of fungi found in or on the tissues of seeds are parasites. However, some seed-borne symbiotic fungi have been recorded. Here a symbiotic fungus which penetrates through the tissues of the parent plant enters the seed and is then transmitted through the seed to the daughter plant, with the result that the symbiotic association is set up once more in the new generation. Here again, therefore, the association is a hereditary one.

Rayner (1913, 1915) recorded the presence of a symbiotic fungus which penetrated throughout the tissues of the plant, and into the seeds of *Calluna vulgaris*, and also in other members of the Ericaceae. A similar condition is known in *Lolium* and was described in great detail by Freeman (1903), though here the connexion between the fungus in the root and that in the seed has not been fully elucidated. The degree of penetration of the fungus in *Calluna* and *Lolium* is different, however. In the former it does not penetrate beyond the testa, but in *Lolium* it enters the embryo and penetrates the growing point, often approaching to within two cells of the tip. Bose (1943, 1946) records the presence of a species of *Phoma* in *Casuarina equisetifolia* which penetrates through the cells of the root, stem, and flower. It occurs in the pericarp wing of the fruits and in the seed-coats. Here, as in *Calluna*, the fungus infects the radicle as it emerges on germination. Thus, Bose states, 'every plant is a dual organism, harbouring a fungus in its seed, so that the symbiotic relationship becomes hereditary'.

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[1] **Part of a thesis approved for the degree of Doctor of Philosophy in the University of Wales.**

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In all these cases when the seed is dormant there is a corresponding dormancy on the part of the fungus. Then the seed germinates, and the growth of the seedling is accompanied by a corresponding renewal of activity of the fungal mycelium, which results in the regular infection of the tissues of the seedling. The fungus passes from plant to plant through succeeding generations. It appears that this method for the propagation of the species and the setting up of a new symbiotic relationship is as successful as the spore method of reproduction, with the consequent renewal of infection of each plant by the fungal mycelium.

The higher plant too seems to depend on the presence of the fungus to complete its reproductive cycle. Rayner (1915) stated that germination and development in *Calluna* are bound up with the infection of the seedling by the fungus from the testa. Freeman (1903) showed that the grains of *Lolium* are incompletely developed in the absence of the fungus. De Jongh (1938) investigated the bacterial symbiont in the seeds of *Ardisia crispa*, and found that the presence of the bacterium in the seed was an essential factor for successful germination. He used the term 'cyclical symbiosis' to illustrate the relationship between the seed-borne symbiont and the plant.

The term 'mycorrhiza' first used by Frank in 1885 means fungus root, and should only be applied to those plants which contain a fungus of a non-parasitic nature in the roots. The term, however, is often loosely used and covers the association between higher plant and fungus, where the fungus spreads beyond the root, into the stem and floral organs and eventually into the seed, from where it reinfects the germinating seedling. To this type of association de Jongh's term *cyclical symbiosis* will be used.

The members of the family Cistaceae, like the Ericaceae, seem to be characterized by the presence of a fungus in the seed-coat. It was known that *Helianthemum chamaecistus* also possessed an endophytic fungus in the roots, and it was thought that the fungus might play a part in the life-cycle similar to that seen in the Ericaceae. Is this seed-fungus a cyclical symbiont? Is the relationship a hereditary one, and is the plant, as Bose suggests in *Casuarina*, a 'dual organism'?

MATERIAL AND METHODS

The greater part of this investigation on these seed-borne fungi in the Cistaceae was carried out on *Helianthemum chamaecistus* Mill. This plant is a calcicole, and occurs frequently on warm dry chalky soils in the south and south-west of England. Plants were examined for the presence of the fungus in the seed and in the root, and in all cases it was found to be present in both these regions. Material was examined while fresh or fixed with formalin-acetic-alcohol (Johansen, 1940). From the outset the greatest difficulty was experienced in obtaining a staining technique which would adequately differentiate the fungal hyphae from the cell contents. A great amount of tannin is present, and this makes it difficult to see the fungus as these tannin deposits tend to pick up the fungal stains. The most successful stain combination was found to be thionin made up in aqueous phenol, with Orange G as a counter-stain (Stoughton, 1930). This results in the fungal cytoplasm appearing purple, while the cell cytoplasm stains blue. Fresh material was stained by the lacto-phenol cotton-blue technique.

The distribution of tannin was followed by staining fresh material with 10 per cent. ferric chloride, or by fixing in an aqueous solution containing 5 per cent. formalin and 10 per cent. ferrous sulphate for 24 to 48 hours; this fixed material was washed, dehydrated, and sectioned but not stained as the iron compound both fixes and stains the tannin.

Seedling tissues were examined by the 'squash' method, and were stained with cotton-blue, or with a modification of the iron-alum-haemotoxylin method suggested by Cook (McLean and Cook, 1941).

DISTRIBUTION OF THE FUNGUS IN THE MATURE PLANT

When the dry seed is placed in water a thin transparent coat is seen surrounding the dark brown testa. This coat adheres to the testa in the dry state, and therefore cannot be observed, but it swells up immediately on contact with water. Examination shows that it is made up of large cells containing numerous starch grains. The interesting feature of this coat, however, is the fact that it is permeated by a septate and branching fungal mycelium (Pl. VII, Fig. 1). Since the fungus is found in the outer coat of the seed, the root of the germinating seedling will, in all probability, become infected with the mycelium as, or soon after, it emerges. The adult plant which showed this infection in the roots did not appear to be diseased in any way, and it was thought, therefore, that the fungus might be symbiotic. Does the fungus in the root bear any connexion with the one found in the seed? If it is the same one, does it enter the seedling on germination and penetrate through the plant, entering the growing points and finally the floral organs and the seed itself? Is this in fact a cyclical fungus which passes the whole of its life in the plant and is transmitted from one generation to another through the seed?

The term 'host' will be used to describe the higher plant, and the fungus will be said to 'invade' or 'infect' the cells. These terms do not imply that the fungus is in any way considered as a parasite.

The distribution of the fungus was followed in the root, stem, and leaf of the plant, in the vegetative and floral meristems and in the flower itself. Any changes in host and fungal tissues were noted, together with any variations in intensity and in the form of the fungus throughout the year.

Root

The distribution and general appearance of the mycelium in the root tissues were examined at all periods of the year. In general the roots of *H. chamaecistus* are long and have very few branches. When the lateral branches begin to develop and break through the parent cortex in March or April, two distinct types of branch root can be seen. Some are long and slender, while others are extremely short and tuberous. To the former the term *long root* will be given, and to the latter *tuberous root.* It is usual to find a number of these tuberous roots developing together; then there may be an expanse of root which bears only long roots.

Tubercles similar to those seen in *H. chamaecistus* have been recorded by Rivett (1924) in *Arbutus unedo.* She states that these root tubercles are arrested secondary and successive laterals of the season's growth, and she shows that the arrest of growth is due to the invasion of the tissues by the fungus from the main root.

It is best to carry out a preliminary study of the distribution of the fungus in the roots in the summer months. In the 1-year-old long root, septate branching fungal hyphae can be seen permeating the cortical cells. They enter and leave the cells at the corners. At this period the great majority of the hyphae are found in the cells, and not in the intercellular spaces. The hyphae appear to be normal and show no signs of any digestion, as is so often the case in the endophytic fungi. The infection seems to be much denser in certain regions of the cortex than in others, but this difference in the density of infection in various regions seems to be quite haphazard. McLennan (1926) states that in *Lolium*, also, infection is not universal for each individual root; some are devoid of the fungus for their entire length, while others show infected patches alternating with uninfected regions. The endophyte of *H. chamaecistus* is most prolific in the middle layers of the cortex, and found very infrequently in the outer layers or in the epidermis. Penetration into the stele appears to be prevented by the thick deposit of tannin-like substances in the cells of the innermost layer of cortex and in the endodermis. McDougal and Dufrenoy (1944) showed that the distribution of the endophyte in the cortex of pines does not extend beyond the endodermis in which catechol tannins are formed. Bose (1943) demonstrated that there was a close connexion between the presence of tannin and the activity and spread of the endophytic fungus in *Casuarina.* In *H. chamaecistus* the endophyte, however, has been observed in the vascular tissues. Actual penetration of the fungus from the cortex, through the tannin-filled endodermal cells into the stele has not been seen, and it is thought that these hyphae seen in the vascular parenchyma, and sometimes in the xylem and phloem cells, have penetrated here by an upward growth from the vascular regions of the young root in the previous year before there was much deposition of tannin in the cells.

It is at this period, when the fungal mycelium is in an active state in the cortical cells of the 1-year-old long root, that branch roots arise. As these roots pass out they may or may not become infected with mycelium from the parent cortex; this obviously depends on whether the fungus is present in those regions in which the root arises. If it is present, it surrounds and eventually penetrates into the cells of the young root before they break through the epidermis. In many cases, however, there is no fungal mycelium in this region, and the young root does not become infected. This uninfected branch root breaks through the parent cortex, and growth is normal and a *long root* is formed. This does not mean that this root remains permanently uninfected. As the fungus increases its activity during the summer months, this long root will eventually become infected by branches of the fungal mycelium from the parent root which will penetrate easily and rapidly through the mature, vacuolated cells. The branch root which is surrounded by fungal hyphae in the parent cortex, and is infected before it reaches the exterior, is destined to become a *tuberous root*. The cells increase greatly in size, and except for a small number of cells near the tip, they appear to lose altogether their meristematic activity. This means that there is very little increase in length, but there is a considerable swelling of the root as a result of the increase in size of the individual cells. These grow until they are about 40 μ across, whereas a normal long root cell at the same stage in development is only 16 μ across.

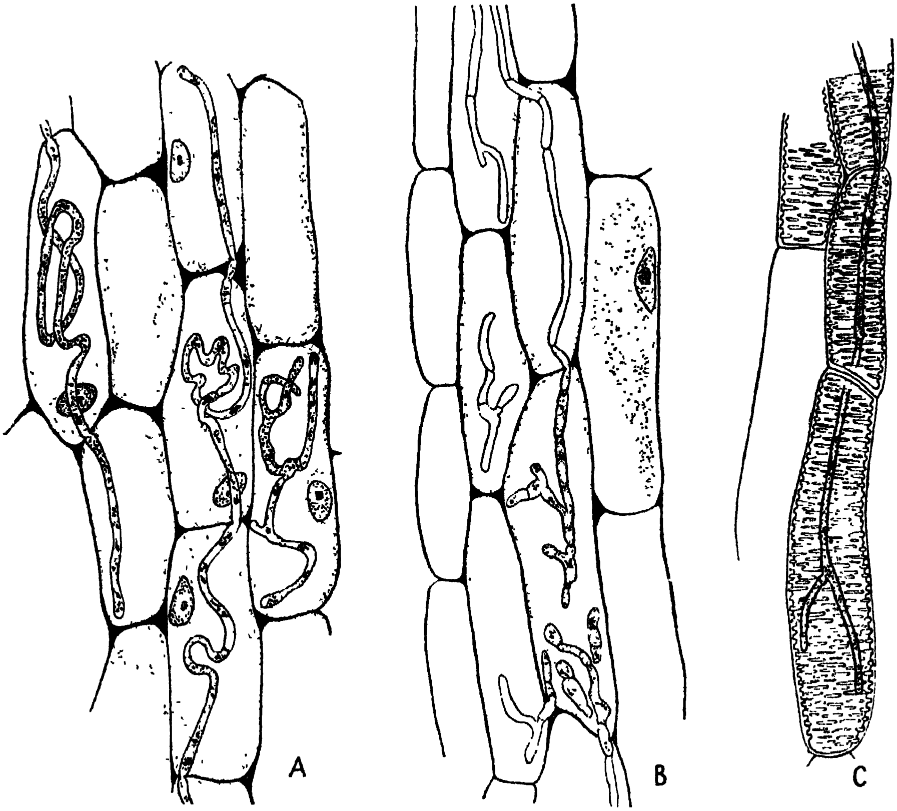
Rivett states that in *Arbutus unedo* tuberization is the result of the successful early establishment of the ectotrophic condition which prevents the continuance of normal root growth. She says that normal laterals show only a few hyphae applied to their surface as they break through the cortex: these roots are the 'straight, long-growing variety' and they continue their growth. Where the hyphae are abundant they invest the root and prevent growth, with the resulting formation of tubercles. In *H. chamaecistus* the formation of long or tuberous roots is not dependent on the density of ectotrophic growth, but is the result of the density of *endotrophic* growth in the parent root at the point at which the branch arises.

It appears that the presence of the fungi in the cortex around the young root, and the consequent infection of the cells at this very early stage, in some way disturbs the metabolism of these cells. They cease to divide, but still increase in size. The increase in size of the cells may be the result of a secretion of certain substances from the hyphae in the parent cortex at the point at which the root arises. It appears that very young cells are incapable of setting up a balanced symbiotic association and that the secretions from the hyphae may disturb the normal activities of the cell. It is rarely that we find mature cells (in which alone we find active fungal mycelium) side by side with meristematic cells; there is usually a gradation from one to the other. It is rarely, therefore, that active mycelium in mature cells will be in contact with meristematic cells. One case of this, however, is seen in the formation of branch roots. That fungal hyphae do upset the normal activities of a meristematic cell is deduced from the fact that branch roots grow normally if there are no hyphae in the cortex around them. The increase in size of the cells, and the consequent tuberization of the root, reminds one of the effect of various growth substances on the cells of roots in particular.

DISTRIBUTION AND CONDITION OF THE FUNGUS IN THE ROOT AT ALL PERIODS OF THE YEAR

During the summer months the mycelium appears to grow very rapidly in the 1-year-old long roots, and extends into the new laterals. During July and August numerous changes take place in the form of the hyphae in the plant, and more particularly in the balance between fungus and host.

The hyphae during this time are extremely vacuolate and have few cross-walls; they branch extensively and form coils in the cortical cells (Text-fig. 1, A). This coiling (Pl. VII, Fig. 2) appears to be followed by the digestion of the hyphae in the cells. The *digestive* process occurs mainly in the 1-year-old long roots and in the tuberous roots. There is very little digestion in the new long roots. Digestion in *Helianthemum* does not seem to be comparable to the clotting and disintegration of the endophyte which is recorded in other endophytic fungi (Rayner, 1915). The hyphae increase in width until they are about twice their normal size, and the hyphal membrane appears to become much thinner. A diffusion of soluble materials may occur from the hypha, through the hyphal membrane, into the cavity of the cell. During the autumn the hyphae in the root are devoid of contents. By this time too the mycelium has broken up into small pieces in each cell, and it is difficult to find the connecting links from cell to cell. During the winter months it is often extremely difficult to find any trace of active fungal mycelium in the cortex



TEXT-FIG. 1. Fungal hyphae in cells of root of *Helianthemum chamaecistus*. A. Coiling and branching of the hyphae in the cortical cells in May. B. Fungus in cortical cells of the long root at the end of the first season's growth. C. Fungal hyphae in xylem element in winter.

of the 1-year-old long root, and the swollen empty hyphae appear to have been completely digested by the host cell. The mycelium, however, appears to be almost unchanged in the current year's long root (Text-fig. 1, B), and it is from here that the new roots will be infected as they arise in the spring and summer of the following year. The mycelium in the intercellular spaces of the cortex of the long root is not digested, but remains in the active hyphal state. This is also the case in the vascular tissues, where septate and branching fungal hyphae can be seen in the xylem elements during the winter months (Text-fig. 1, C).

In the tuberous roots many changes take place during the late summer and autumn. The mycelium, which was mainly intracellular, tends to become more and more intercellular especially at the tip of the root, so that one sees two fairly clearly defined regions. The first is the region with great intracellular infection nearest the main root, and the other is a region of much intercellular mycelium and only slight intracellular infection near the tip. The digestive process is much more drastic in the tuberous roots, and the intracellular hyphae break up into numerous small portions, swell up, and lose their contents. The intercellular hyphae are not affected by this process and the tendency is for the mycelium to grow out through the intercellular spaces of the cortex in between the epidermal cells and thus form a mat of ectotrophic growth on the surface. At the tip of the root, where there is much intercellular mycelium, there is a rapid growth of fungal hyphae towards the exterior, where they branch and form a thick dense mat on the outside of the root. The hyphae here are so interwoven that they give rise to a pseudoparenchymatous layer outside the cortex and epidermis. There are now two even more distinct regions in the tuberous root (Text-fig. 2):

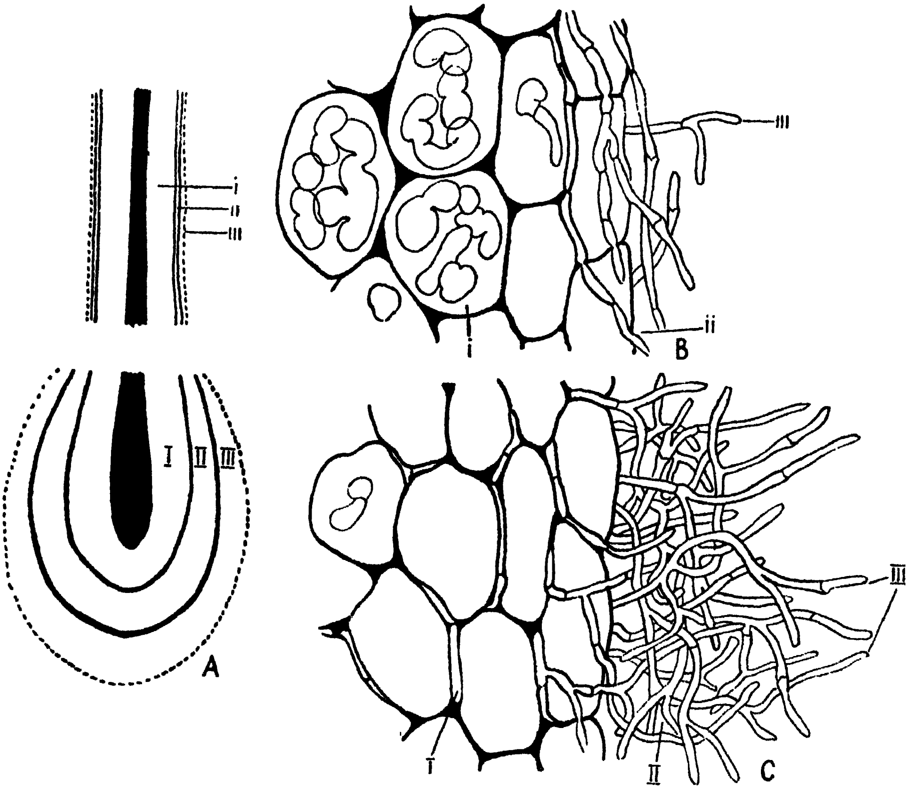
(*a*) Near the main root there is a region of great intracellular infection coupled with only slight ectotrophic growth. The hyphae in the cortical cells have become greatly swollen, they have a very thin membrane and no contents (Text-fig. 2, B).

(*b*) Towards the tip there is a region of great intercellular infection coupled with dense ectotrophic growth. There is only slight intracellular mycelium, and this is in the characteristic digested state. The intercellular mycelium, however, is still in the hyphal state, is full of cytoplasm, and possesses extremely thick walls. It passes out between the epidermal cells and forms the dense mat of interwoven hyphae of a pseudoparenchymatous nature on the surface. From this strands of hyphae pass outward into the soil (Text-fig. 2, C).

It is easy to pick out these tuberous roots in September by their swollen appearance and dark colour: the ectotrophic hyphae can be seen quite clearly with a hand lens. During the winter months the ectotrophic growth is cut off, firstly by the production of a thick layer of cork on the outside of the tuberous root, and secondly by a zone of tannin-filled cells inside this.

The mycelial cycle in the roots can be summarized in the following way:

1. During the spring there is an active fungal mycelium in the 1-year-old long roots. Branch roots arise and develop into tuberous or long roots, depending on whether they become infected or not as they pass through the parent cortex.



TEXT-FIG. 2. Diagram to show type of infection in tuberous root in September in *Helianthemum chamaecistus*. A. L.S. of tuberous root showing the two main regions. B. Region with great intracellular and slight intercellular growth. C. Region with intercellular infection and dense ectotrophic growth: i. cortical cells full of swollen, empty distorted hyphae; I. cortex with unaltered intercellular mycelium; ii. thin mat of interwoven hyphae; II. dense mat of interwoven hyphae forming pseudoparenchymatous layer on the surface; iii. extremely sparse ectotrophic growth; III. loose web of innumerable ectotrophic hyphae.

2. During the summer the long roots increase in size and produce more laterals. The fungal hyphae in the parent cortex are active and they eventually penetrate through the cells of the new long roots.
3. Digestion, preceded by coiling and branching of the hyphae, occurs in late summer in the 1-year-old long roots, and in the tuberous roots. It does not occur in the current season's long roots. The hyphae at the tip of the tuberous root pass into the intercellular spaces and form a dense ectotrophic growth on the surface.

4. In the winter there is very little sign of active mycelium in the old roots but it is still found in the young long roots. The ectotrophic hyphae of the tuberous root are cut off by a layer of cork and tannin cells.
5. In the following spring the now 1-year-old long roots with active mycelium act as a source of infection for active endophytic growth in the newly formed roots.

Stem

It is extremely difficult to find any trace of fungal infection in the stem. The outer and inner cortical cells contain heavy deposits of tannin-like substances, but the middle layers of cortex are devoid of them. The pith also contains a high percentage of tannin-filled cells. These deposits take up the normal fungal stains, and for some time the tannin deposits were taken for digested masses of fungal hyphae in the cells. The branching Holmgren canals are very similar to the masses of partly digested hyphae seen in endophytic mycorrhizal infections.

It is thought that fungal hyphae penetrate through the young plant from the root upwards through the vascular parenchyma of the stem, in and between the cells of the cortex, and sometimes in the xylem and phloem elements. As the cells mature it is difficult to find any mycelium in the stem tissues, but inter- and intracellular mycelium has been observed in the cortex and pith. Active fungal hyphae can also be seen in some of the phloem cells, and in the vascular parenchyma just behind the apical meristem. It seems that the fungus keeps pace with the growth of the apex and enters new tissues as they arise. Fungal hyphae extend upwards in the vegetative apical meristem as far as the fifth and fourth pair of leaf-bases, and from here they extend into the leaves themselves. The fungus does not penetrate into the meristematic cells, and the endophyte was only found in those cells in which vacuolation had commenced. The fungus is most dense in the vascular region at the node. When the axillary buds develop the fungus spreads into the new tissues, and in the small axillary shoot the condition seen in the apical meristem is repeated.

Active fungal growth only occurs in the younger regions of shoot and root, while in the older regions it appears to be absent. There is no apparent connexion between the mycelium in the root and shoot of the adult plant, although shoot infection originally arose from the mycelium in the root. This is probably the result of the digestion and disappearance of the fungi of the older regions of the root so that the connexion between root and shoot is severed.

Leaf

The fungus can be seen in the leaf in the vascular parenchyma in a greatly attenuated form, and it extends also into some of the mesophyll cells. The fungus was never observed in the palisade tissue.

Floral organs

The fungus in the vascular parenchyma extends to the base of the floral meristem. The rudiments of the flower are completely laid down before the fungus penetrates into the receptacular tissue which has now become vacuolate.

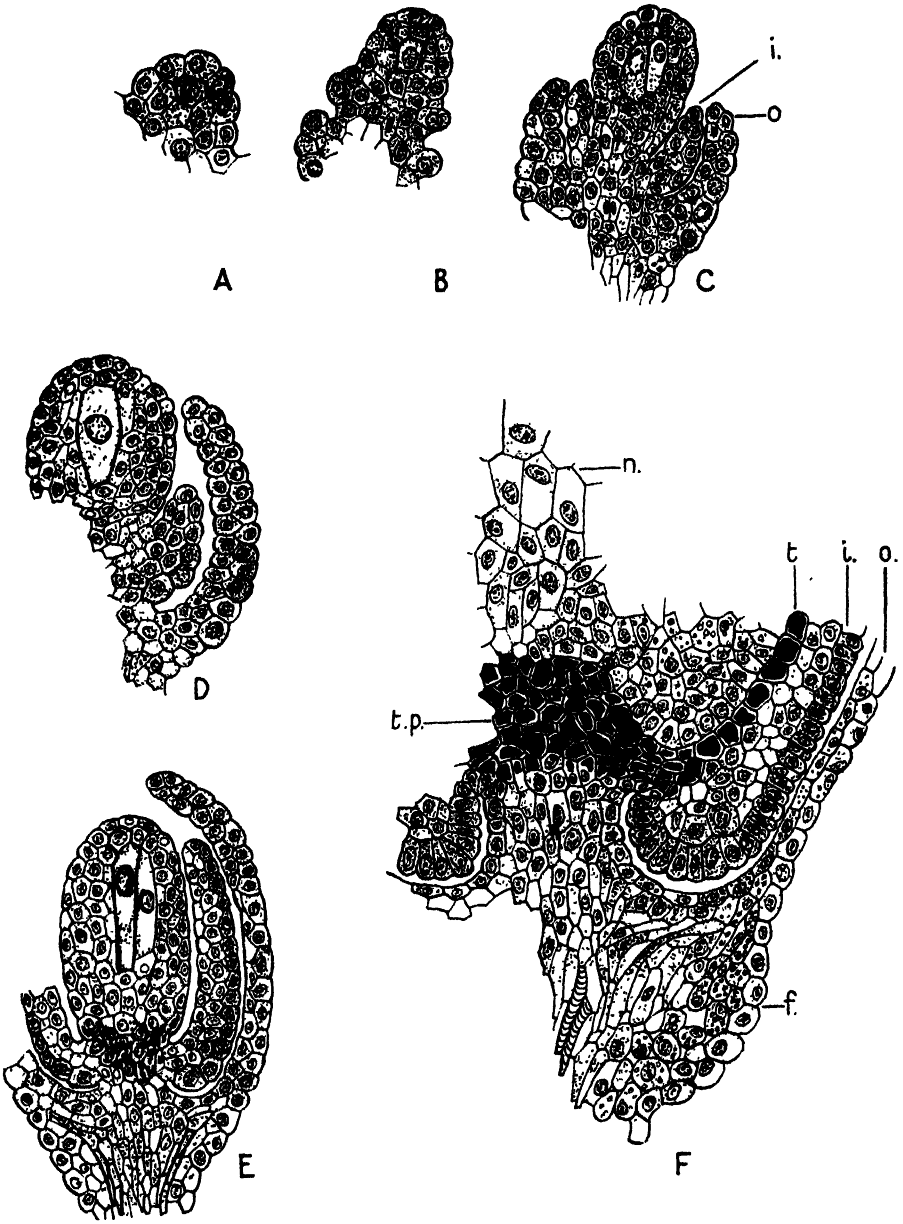
INFECTION OF THE SEED BY THE FUNGUS

The floral bud begins its development as a cone-shaped mass of tissue on the floral apex. These cells are full of cytoplasm and there is no sign of fungal infection. Five sepal and five petal primordia arise on this cone, followed by four tiers of stamen primordia and finally the carpel initials. In the first stages the carpels are crescent-shaped primordia with the concave sides facing the apical meristem of the receptacle. As the growth of the carpels proceeds, lateral veins from the midrib extend in all directions to ramify within the tissues of the carpellary wall, thus laying down the means of supplying the ovule, and later the seed, with nutritive materials, and in this case acting as a means whereby the fungal mycelium may pass rapidly into all parts of the ovary. The ground tissue of the carpel shows an intercellular system of air spaces, and the cells often contain large crystals of calcium oxalate.

Development of ovule

A small protuberance of meristematic cells is formed on the placenta. Integuments arise as rings of tissue around this ovule primordium (Text-fig. 3, A, B, C). Inner and outer integuments develop almost simultaneously, but the latter integument grows more rapidly than the former. It is made up of two layers of cells, while the inner integument is three-layered. These cells of the outer integument grow rapidly; development of the inner integument is slower and very complex. Some interesting and characteristic features occur at the chalazal end of this orthotropous ovule. The basal cells of the outer layer of the inner integument increase in size and multiply rapidly, but instead of producing an upward growth from chalaza to micropyle as is normally seen, these rapidly formed cells produce a bulge of tissue in this region (Text-fig. 3, D, E). This bulge is produced so rapidly and is so great that there is an invagination of tissue, the outer cells being pushed inwards and upwards into the nucellar tissue, at the base of the ovule (Text-figs. 3, F; 4). Thus a ring of invaginated, extremely large columnar cells is formed in this region. These cells do not completely block the entrance from the funicle to the nucellar tissue; they leave a column of elongated undifferentiated parenchymatous tissue, about five cells across in between. This development of the outer layer of cells of the inner integument is a characteristic feature of the ovule of *H. chamaecistus*. The layer is easily recognized by its closely packed, radially placed, thick-walled columnar cells. The inner two layers of cells of this integument remain unmodified, as do the cells of the outer integument.

When the rudiments of the flower have been laid down, rapid growth of the filaments of the stamens and of the petals occurs and the flower opens.



TEXT-FIG. 3. Development of ovule of *Helianthemum chamaecistus*. A. Ovule primordium on placenta. B. Inner and outer integument initials. C and D. Development of the three-layered inner and the two-layered outer integument. E. Development of 'bulge' at the base of inner integument. F. Base of developing ovule showing tannin-filled cells. *i.* inner integument; *o.* outer integument; *t.* tannin layer; *t.p.* tannin plug; *n.* nucellus; *f.* funicle.

After this, all growth is the result of increase in cell size, accompanied by vacuole formation. It appears that this is the stage when the fungus enters the flower parts, and it can therefore play no part in the nutrition of the developing flower.

Penetration of the fungus into the seed

The fungus appears to penetrate rapidly through the vacuolated parenchymatous cells and through the elongated cells associated with the vascular tissue. It passes into the vascular parenchyma of the receptacle, and from here into the ovary wall. If strips of the inner epidermis of the ovary are examined the fungus will be seen penetrating through the cells. It passes from the carpellary tissue, through the placenta to the funicle of the ovule, where it is found in the vascular parenchyma, the cortex, and occasionally in the xylem elements.

If the fungus can penetrate as far as the funicle it should be able to pass from here into the ovule. If the fungus can enter the ovule, and therefore the seed, its entry into the next generation is certain.

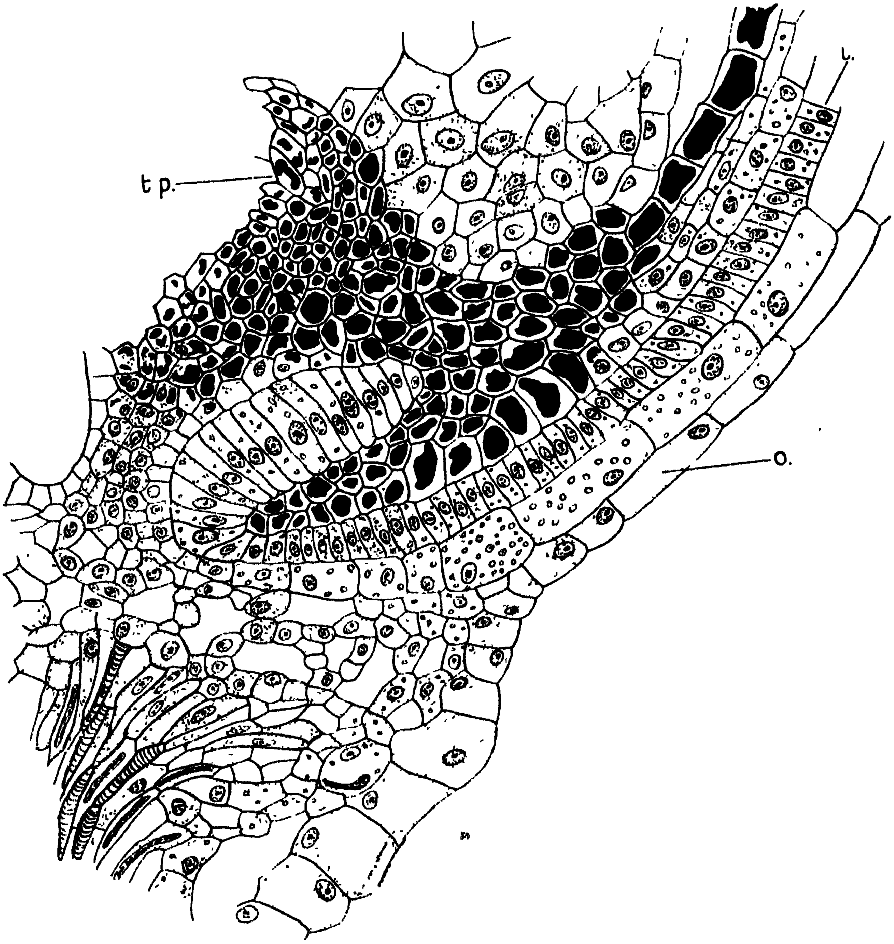
In the adult plant a balance is set up between the fungus and its host. It is debatable whether the minute embryo could set up this balanced relationship, and whether it could prevent the fungus from parasitizing it. The seed provides another example of mature cells being in contact with meristematic cells. When this occurred in the root, the meristematic cells were adversely affected by the presence of the fungus in the mature cells, and their metabolism was disturbed and cell-division ceased. However, the fungus never enters the seed proper; it only penetrates through the cells of the outer integument.

During the maturation of the ovule various modifications of the tissues occur. The column of cells at the chalazal end of the ovule (the column which is surrounded by the invaginated ring of columnar cells of the inner integument) is the first region to show signs of these changes. Each cell becomes filled with a tannin-like deposit. A dome-shaped area of nucellar cells immediately anterior to this column also becomes filled with tannin, as does the complete outer layer of nucellus cells. This means that the nucellus tissue, and in time the embryonic tissue too, is surrounded by an unbroken layer of tannin-filled cells. These changes mean that the base of the ovule is 'plugged' with an impenetrable tannin-filled barrier. The fungus which fills many cells of the funicle cannot pass into the ovule tissue or into the developing embryo (Text-figs. 4, 5).

The fungus, however, *can* pass from the funicle into the cells of the outer integument. Having entered these cells, penetration of the fungus into the tissues of the ovule is prevented firstly by the presence of the tightly packed thick-walled columnar cells of the inner integument, and secondly by the tannin layer inside this. The fungus could enter the embryonic tissues if it were present in the pollen grain; a great number of grains were examined for the presence of the fungus, but always with negative results.

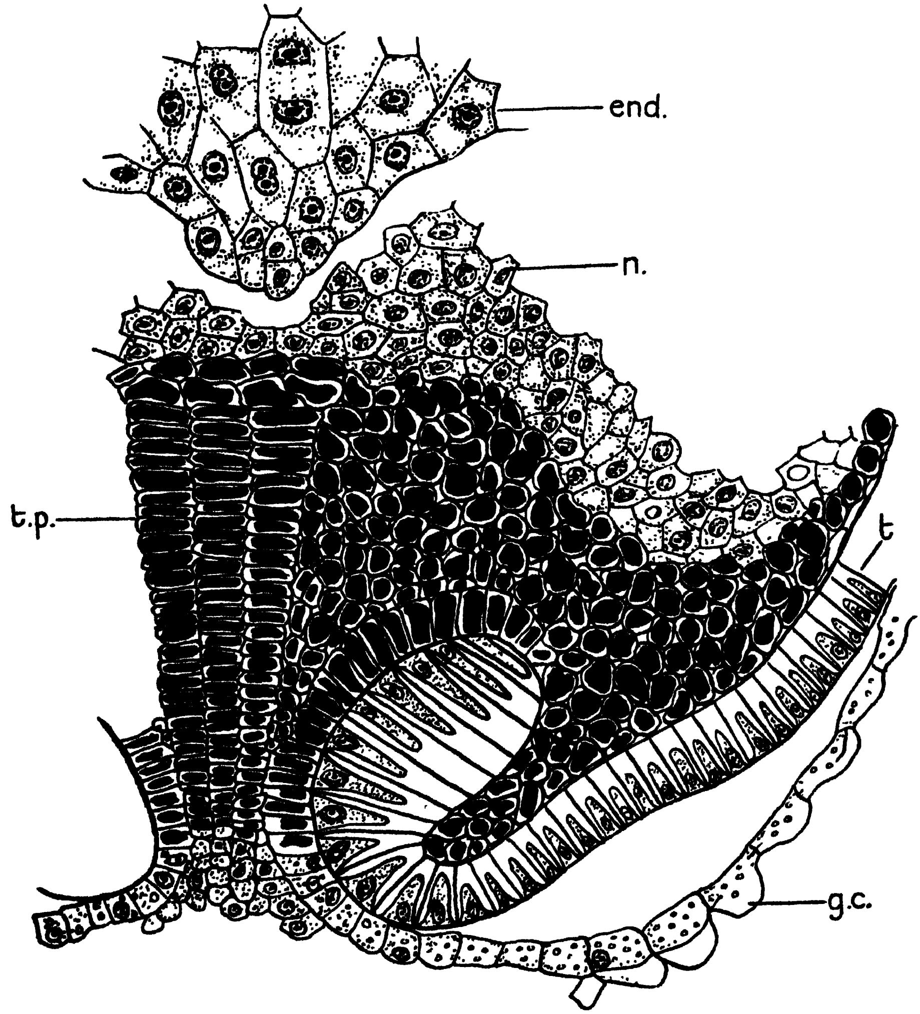
Maturation of the seed

As the seed matures, the outer cells of the outer integument become devoid of contents, and all that can be seen is the nucleus which clings very closely to the wall. The inner cells are still full of starch grains. During maturation of the seed much water is given off, and it is thought that at this stage the wall between the two layers of cells collapses, giving the single-layered coat made up of cells full of starch grains and containing the fungus resting on the thick-walled columnar cells of the inner integument (Text-fig. 6, B).



TEXT-FIG. 4. L.S. Base of fully developed ovule of *Helianthemum chamaecistus*, showing the fungus in the vascular parenchyma of the funicle. Note the columnar cells of the outer layer of the inner integument. *i.* inner integument; *o.* outer integument; *t.p.* tannin plug.

In the mature seed the outer layer of the inner integument forms the important part of the seed-coat. These tightly packed columnar cells become thickened along the inner and along the four lateral walls, but the outer wall remains unthickened, and the characteristic U-shaped thickening results. This layer becomes deeply pigmented and forms the hard brown testa of the seed. The inner two layers of cells of this integument disintegrate completely. When the seed is dry, the outer coat, which contains the fungus, adheres very closely to the thick-walled columnar cells which constitute the seed-coat. When the seed is soaked in water this coat swells up and forms a thin, almost transparent film around the seed, with a small aperture at the micropylar and chalazal ends where the layer is obviously not complete. This is the layer to which the term *gelatinous coat* has been given.

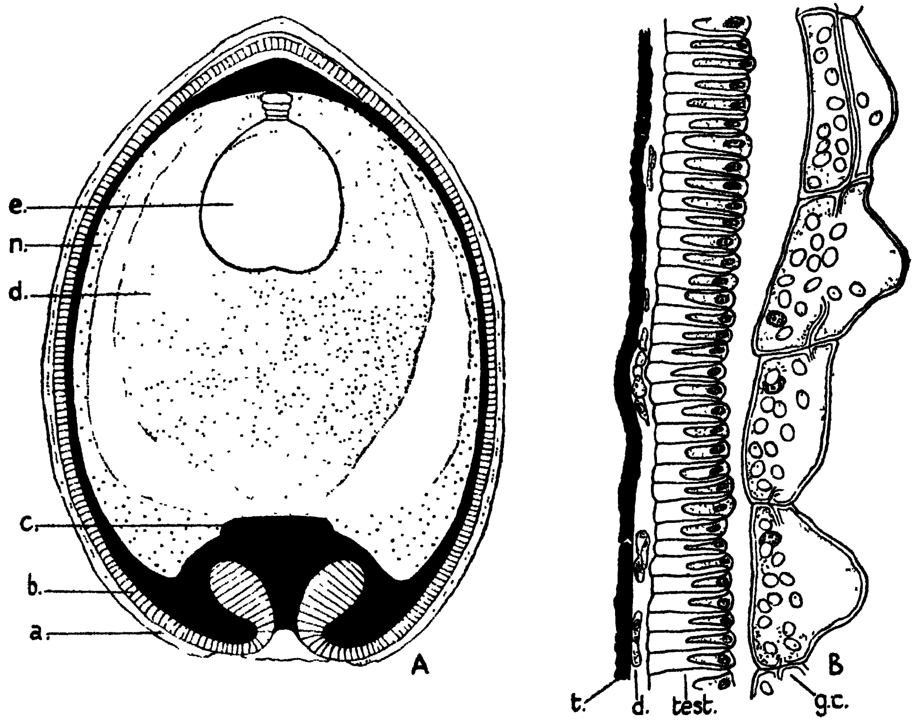


TEXT-FIG. 5. L.S. Base of seed of *Helianthemum chamaecistus*, showing the density of the tannin plug. *end.* endosperm; *g.c.* gelatinous coat; *n.* nucleus; *t.p.* tannin plug.

In a section taken longitudinally through the mature seed the following characteristic features can be seen (Text-fig. 6, A):

(*a*) An outer layer of unthickened unmodified cells, full of starch grains through which the hyphae ramify (the gelatinous coat).

(*b*) The testa, made up of thick-walled radially placed columnar cells with the characteristic invaginated ring at the chalazal end.



TEXT-FIG. 6. L.S. through the seed of *Helianthemum chamaecistus*. A. Plan of seed (for legend see text). B. L.S. through the testa and the gelatinous coat. *d.* disintegrated cells of the inner two layers of the inner integument; *g.c.* gelatinous coat; *n.* nucellus; *t.* tannin layer; *test.* testa.

(*c*) A complete layer of tannin-filled cells around the endosperm, one cell thick at the sides and apex but made up of many cells at the base, which seems to form an impenetrable barrier against the entry of the fungus.

(*d*) The endosperm tissue, with bi- and tri-nucleate cells.

(*e*) The embryo itself at the micropyle end of the seed.

The only part of the seed in which the fungus is found is the thin-walled gelatinous coat. It is clear also how the development of peculiar features, characteristic of this genus, prevents the penetration of the fungus into the tissues of the seed. ***The embryo plant, with its surrounding endospermic tissue, is the only stage in the whole life-cycle which does not show a symbiotic relationship between plant and fungus*** (cf. *Calluna* and *Casuarina*).

The fact that the fungus is found in the outer coat means that the embryo plant can, on germination, become infected as the root breaks through this coat.

This elaborate mechanism, which is apparently directed towards preventing the fungus from penetrating the embryo, ensures that fungal infection, and the consequent setting up of a symbiotic association, does not take place until the minute plant is capable of maintaining such an association.

GERMINATION AND GROWTH OF THE SEEDLING AND ITS REINFECTION BY THE FUNGUS FROM THE GELATINOUS COAT

It is desirable that all experiments with seedlings should be carried out under completely sterile conditions. One can then be certain that one is dealing with the fungus from the seed-coat, and not with saprophytic forms that may cling to the testa or may enter the root from the medium in which the seedling is growing.

Sterile seeds were obtained by dissecting open an almost ripe ovary under sterile conditions and removing the seeds into sterile containers. This method is the one described by Rayner (1915) in her work on *Calluna vulgaris*.

The greatest difficulty met with in this work was the fact that the percentage germination was often nil. McLennan (1935) points to a similar difficulty with seeds of *Epacris impressa*, and Rayner (1929) met with the same problem in experiments on the mycorrhizal relationship in the genus *Vaccinium*.

The seeds of *H. chamaecistus* are extremely hard, and to induce germination the method of puncturing each seed individually was adopted. This was done under sterile conditions, and care had to be taken to see that the gelatinous coat was not removed during the process. The punctured seeds were set on a variety of media, and the germinating seedlings were examined for the presence of the fungus. The gelatinous coats were also removed from the seeds at various stages in germination to observe the growth of the mycelium.

When the seeds are placed in, or on, the culture medium, the gelatinous coat swells up immediately. If this coat is removed a few days after germination, sculpturing of the starch grains is very marked, and this suggests that the starch is being digested. Whether this is one of the functions of the fungus is not certain: the fungal mycelium, however, has grown to a considerable extent. The root breaks through the ruptured portion of the testa within a few days of setting the seed. An interesting feature of this root is the absence of root-hairs. This lack of root-hairs has been recorded in other mycorrhizal plants; Rayner (1915, 1933) states that they are absent in *Calluna vulgaris* and in *Citrus*, and it raises the question whether this lack of root-hairs has any bearing on the mycorrhizal association. The root possesses an extremely long root-cap, the cells of which extend initially from the junction of hypocotyl and radicle to the tip of the root. In culture the cells of the piliferous layer and the outer layers of the root-cap are seen to come off in strips. These strips sometimes break off completely, but normally they remain attached to the plant, and give the appearance of multicellular root-hairs. The whole cortical tissue often becomes broken up by transverse breaks from piliferous layer to stele. It appears that the cells of the epidermis and cortex possess walls in which the calcium pectate layer is harder than is normally the case. This may be the result of the calcicolous habit. Normal growth of the cell is the result of the stretching of the wall when the cell becomes turgid. In this case, however, the walls are too deeply impregnated with calcium pectate, and are so hard that stretching does not occur, and as a result there is no increase in size of the cell. The failure of production of root-hairs can probably be attributed to the same cause. The elements of the stele in the same region elongate rapidly, however, and so we get a condition in which there is rapid growth inside the root, correlated with little or no growth of the outer tissues. This must result in the breaking of the outer cells; sometimes the epidermis only is affected, but at times many layers of cortical tissue are broken up too. The possible significance of this phenomenon will be discussed later.

When the seedling root is examined for the presence of the endophyte, small strands of hyphae can be seen in the cells of the cortex. Infection must have taken place from the seed. This infection of the young root by the mycelium in the gelatinous coat occurs soon after the radicle has emerged. Before the hyphae grow out and penetrate the root, they grow and increase in length at the expense of the starch grains in the cells. When the mycelium grows out of the coat, the root is already a few millimetres long; this means that the region of the root which comes in direct contact with the hyphae is that in which tissue differentiation has commenced. The pro-meristem cells do not appear to come in contact with the mycelium, and it is the region immediately behind the pro-meristem which becomes infected by the fungus from the coat. It is extremely difficult to follow the hyphae in the seedling; first examination almost invariably brings negative results. The hyphae in the gelatinous coat are septate, but when they enter the root septa become rare or absent. They penetrate the cell-walls, and where this happens there is a slight constriction. The fungus is mainly intracellular, and it penetrates from cell to cell, entering new cells at the corners. From the cortex it passes into the elongated thin-walled pro-cambial elements. As the first phloem and xylem cells are differentiated from the pro-cambial cells, the hyphae gain access to these tissues. The protoxylem and protophloem cells are slender and long, and possess thin cellulose walls: these cells are used by the fungus as a means of rapid spread through the plant. As the pro-meristem of the root divides and as new tissues are formed, the fungus spreads into these regions as they are differentiated. Thus, from the original point of penetration, the fungus now extends into all new root tissues as they are formed. Initial infection took place at a point just below the junction of root and hypocotyl. From here the fungus spreads into the hypocotyl and eventually into the plumule. In the shoot it extends as far as the region of tissue differentiation just behind the promeristem at the apex. As in the root, it keeps pace with the growth of the apical meristem and enters new tissues as they arise. The hyphae usually pass into the leaf with the phloem parenchyma, and they are thus found in the dorsal areas of the leaf.

In this way the fungus spreads throughout the seedling and thereby gains access to all tissues of the plant as they are formed. In the older tissues of the root the fungus will, at a later stage, undergo a process of digestion, and the hyphae in the mature vascular elements begin to degenerate. In this way contact between root and shoot mycelium is broken and in the adult plant it is difficult to find any trace of the endophyte in the older tissues. The fungal mycelium remains active in the roots of the seedling throughout the first winter, and it is from the cortical cells in these regions that the young laterals are infected as they arise in the following season. On the whole, digestion of fungal hyphae on a large scale does not occur until the end of the second season of growth.

GENERAL DESCRIPTION OF THE FUNGUS

It is extremely difficult to give a morphological account of the fungus, as its form varies considerably and seems to depend on the type of tissue in which it is found, and on the period of the year. Rayner (1915) stated that the mycelium in the tissues of *Calluna vulgaris* was distinguished from that outside by the smaller diameter of the hyphae. She also pointed to the variation in diameter within the tissues. McLennan (1926) showed that in *Lolium* the walls of the hyphae, which were thick and brown outside the root, were thin and colourless inside, but that the dimensions increased greatly. This variation is seen in *H. chamaecistus* also. In the gelatinous coat of the seed we find a branching septate mycelium, with fairly dense cytoplasm and numerous vacuoles. The hyphae are about 4μ in diameter. These are the hyphae which penetrate the radicle on its emergence. In the root septa are rare, but the hyphae still contain dense cytoplasm and there is very little change in their diameter. In the regions where there is active growth of the mycelium, the hyphae maintain these characteristics. It is in the mature tissues that changes in hyphal form are seen. Partial digestion of the intracellular mycelium in the root results in a considerable increase in the diameter of the hyphae, and the cytoplasm becomes much less dense. The hyphae are gradually emptied of all their contents. The intercellular mycelium does not show this great increase in diameter, although the hyphae now measure 8μ across. The ectotrophic hyphae formed at this period have thick pigmented walls, which do not take up the stains. They have numerous cross-walls and they measure from 5 to 8μ across.

There is no indication of the presence of vesicles, arbuscules, and sporangioles. The fungus has only been seen in the hyphal form: there are no reproductive bodies. It has not yet been possible to grow the fungus in pure culture, notwithstanding the use of a great variety of media.

FUNCTION OF THE FUNGUS IN THE PLANT

Since the classical researches of Frank (1885) much discussion has centred around the relationship which exists between the two components of a mycorrhizal association, particularly in the case of endophytes. Bernard (1904) called the endophyte in the orchids a parasite, and suggested that it attacked the embryo plant at an early stage and persisted in the adult as a chronic, but relatively benign condition of disease. Burges (1936) states that the association between the fungus and the roots of higher plants should be regarded as one of controlled parasitism, as it has no 'mutualistic significance'. He says the fungi are weak pathogens whose activity is curbed by the reaction of the host cells. Later (1939) he states that 'breakdown of the hyphae in the root is probably the most striking phenomenon associated with mycorrhizal infection, and is responsible to a large extent for the separation of mycorrhizal infection from the more usual forms of parasitic invasion'. He goes on to say that breakdown results in the liberation of fungal proteins into the cell and thus may represent a gain to the plant. Against this theory of the controlled parasitic nature of the endophyte, we find a mass of evidence pointing to the symbiotic nature of the fungus. Many workers have suggested the function of *nitrogen fixation* for the endophyte. Ternetz (1907) found that fungi isolated from the roots of Ericaceous plants were able to fix atmospheric nitrogen. Rayner (1915), experimenting with pure culture seedlings of *Calluna vulgaris* inoculated with the endophyte and grown under sterile conditions in media lacking combined nitrogen, found that they were green and healthy, and grew at the same rate as the controls. Neilson-Jones and Smith (1928) cultivated the fungus from *Calluna* and concluded that it could utilize the molecular nitrogen of the air.

Another theory of the function of the fungus suggests that some sort of *exchange of food material* takes place between the endophyte and its host. Many workers suggest that the fungus supplies an enzyme which renders soluble the insoluble materials stored in the cells, while it has also been shown that the host plant may produce an enzyme to bring about the digestion and subsequent utilization of fungal material in the cell. Knudson (1922) suggested that the activating effect of the fungal mycelium in the orchids was due to the production of enzymes which acted upon the insoluble carbohydrates present in the seed tissue. Endrigkeit (1937) also supports this theory and shows that the disappearance of starch from the root cells of the host is a reaction to fungal infection. McLennan (1926) showed that in *Lolium* the fungus itself was digested by the root cells and was used 'as a source of carbonaceous nutriment'. Fat or oil which accumulates in the hyphae is, at a certain stage in the association, given up to the host cell through the disintegration of the sporangiole wall. That this is utilized by the higher plant is evident by its removal from the cells. McDougal and Dufrenoy (1946) suggest, as a result of cytochemical tests on the cortical cells, that carbohydrates, phospholipids, and proteins are metabolized in the hyphae of the fungus, and are then transported to the cortex of the higher plant.

Muller and Schopfer (1937) and Robbins (1939) suggest that symbiosis may be intimately related to the ability of one member of the association to *supply growth factors* to the other. Burgeff (1934) was the first person to work on this line; he suggested that vitamin autotrophic micro-organisms were capable of producing accessory growth factors which were supplied to the host. He found (1936) that the dead symbiont of the orchids was quite as effective in stimulating growth as the living fungus, and he showed that the active principle, normally formed by the fungus, was an accessory growth factor resembling Bios II. The orchids of the *Vanda* group are heterotrophic for this substance at least in the seedling stage. This inability of the plant to synthesize the growth factor is made good by the autotrophic fungus. Schaffstein (1938) showed that the seed of *Phalaenopsis* contained very little vitamin, although the green parts of the mature plant were relatively rich in vitamin. It is thus only during the early stages that the plant requires the growth substance which the fungus can synthesize. Noggle and Wynd (1943) obtained good germination and excellent development of seedlings of *Cattleya* when nicotinic acid was added to the medium, and suggested that this was the factor that the seedling was unable to synthesize, and which under normal conditions was supplied by the symbiotic fungus.

In view of previous work carried out on the function of the fungus in many kinds of symbiotic associations, three main lines of investigation were followed to ascertain the probable function of the endophyte of *H. chamaecistus*. These were the possible fixation of atmospheric nitrogen, exchange of food substances, and the supply of accessory growth factors.

If the fungus is isolated one can study its metabolism under controlled conditions. If this is impossible one has to fall back on microscopic examination and upon a study of the host's metabolism in relation to its symbiont. Attempts must also be made to grow the plant from sterile seeds by removing the gelatinous coat and thus obtain a fungus-free plant. To the medium in which this plant is growing various substances must be added to ascertain whether one or a combination of these substances is able to replace the fungus. In this way it may be possible to deduce the probable function of the fungus in the plant.

Experiments on the possible nitrogen fixation by the fungus

Seedlings grown without the fungus germinate successfully, but growth ceases at a certain point. The plumule does not emerge and no branch roots are formed. Seeds from which the fungus has not been removed develop normally. It appears that the presence of the fungus is essential for successful growth. To ascertain whether the need for the fungus was bound up with the power to fix atmospheric nitrogen, seeds with and without the fungus (i.e. those with and without the gelatinous coat) were grown in a full nutrient solution with an inorganic nitrogen supply, with an organic nitrogen supply in the form of asparagine or glycine, and lastly in a solution from which every source of nitrogen had been removed. An attempt was made to provide a basic solution which included all essential substances for plant growth. In this way it was felt that lack of growth could not be put down to any cause other than the lack of nitrogen supply. The solution used was that recommended by White (1943) for tissue-culture work.

In all cases seeds germinated successfully and reached the stage of production of hypocotyl and cotyledons, and an unbranched primary root. From this point onwards growth differed greatly. The seedlings grown *with* the fungus, on a medium lacking combined nitrogen in any form, failed to continue their development and after some time the root became brown and growth ceased. Germination occurred because the plant was able to rely on the nitrogenous supply in the seed; when this was utilized, the seedling was dependent on an *outside* supply of combined nitrogen; without this no growth occurred despite the presence of the fungus. Seedlings grown without the fungus but with an inorganic or organic nitrogen supply also failed to develop beyond a certain point. Thus a supply of combined nitrogen cannot replace the fungus, and the latter is incapable of fixing atmospheric nitrogen and supplying it to its host.

Experiments on the possible exchange of food between fungus and host

Most of this work was carried out with roots because of the greater intensity of hyphae in these regions. Since etching of the starch grains in the cells of the gelatinous coat had been observed, it was thought that the fungus might play some part in making insoluble carbohydrates available to the plant. Observations were therefore made on the distribution and occurrence of carbohydrates in the cells.

Seedling roots were examined, and it was found that although starch was absent in normal symbiotic roots, in those plants which were grown without the fungus there was quite a heavy deposition of grains in the cells. Older roots were also examined, and where there was dense endotrophic infection there was no starch. Burgeff (1909) showed that in orchids, too, the appearance of an intracellular mycelium coincided with the disappearance of starch from the invaded cells.

The presence of the fungus appears to be correlated with the absence of insoluble carbohydrates. Is the function of the fungus that of supplying the plant with soluble carbohydrates? Does this mean that the fungus can be replaced, as Knudson suggests for the orchids, by supplying the plant with sugars? Experiments on symbiotic growth showed that provision of sugar alone was not enough to bring about successful development, although growth did show some improvement. We can say with some certainty that the fungus benefits the plant as a result of its action on insoluble carbohydrates: this, however, is not its only function. It is probable that the fungus utilizes some of the soluble carbohydrates for its own metabolism.

Tests for the presence of proteins and fats in the cells and hyphae were carried out. There appears to be no relationship between the presence of proteins in the cells and the presence of the endophyte. There is no storage of fats in the cells of the plant.

At the end of the summer shrivelled empty hyphae are seen in some of the root cells which are also devoid of contents. It is likely that soluble substances diffuse through the thin fungal membrane into the cell and are transported directly to the growing parts of the plant. It is obvious that at this period the endophyte is used in some way by the root as a source of nutriment.

Experiments to ascertain whether the fungus relieves a possible avitaminosis in the plant by supplying one or more essential growth substances

In experiments on asymbiotic growth of seedlings, thiamin, biotin, pyridoxine, and nicotinic acid were added alone and in various combinations to the basic medium. It was found that the addition of thiamin (1 in 20,000,000) resulted in successful germination and development of seeds devoid of the fungus, the plumule of the seedling emerging and the primary root branching extensively. Kögl and Haagen-Smit (1936), working on detached pea embryos, showed that thiamin was essential for development and that it markedly affected the growth of the roots. It is root growth which suffers in asymbiotically germinated seeds of *H. chamaecistus*. These results suggest that without the fungus the plant suffers from a lack of thiamin, but that in nature this is made good by the presence of a fungus which is auxo-autotrophic for this substance. It leads us to the opinion that one of the functions of the fungus is to relieve this avitaminosis in the seedling by supplying it with thiamin. Experiments to ascertain whether thiamin was present in the seed and in the mature tissues were carried out and will be described, together with full details of asymbiotic growth, in a later paper. It can be deduced with some certainty from this work that one of the functions of the fungus in *H. chamaecistus* is to provide the plant with thiamin.

Experiments on the stabilizing of the calcium metabolism of the host by the fungus

Under cultural conditions the primary root of seedlings and the branch roots of older plants show a marked stripping of the epidermis, and numerous transverse breaks in the cortex. Phenomena such as these have been recorded in many plants suffering from an excess of calcium in the culture solution (Cormack, 1935), and it is suggested that this is due to the fact that the walls become unduly impregnated with calcium pectate. *H. chamaecistus* is a calcicolous plant and an excess of calcium in the cells is to be expected. There is a heavy deposition of calcium oxalate crystals in the cells, these crystals often being found in association with fungal hyphae. We know that fungi frequently produce oxalic acid as a by-product of their metabolism. Does this fungus give off oxalic acid, which combining with the calcium taken in by the plant, removes the effect of an excess of calcium and stabilizes the calcium metabolism? It is difficult to say whether the oxalic acid in *Helianthemum* is produced by the fungus. It may be formed in other ways. Calcium oxalate crystals, however, are only found in those seedlings grown with the fungus, and it is reasonable to suppose that there is some connexion between fungal infection and the presence of crystals.

Seeds with and without the fungus were germinated in an inorganic medium with calcium and one without, and the seedlings were examined for the presence of excess calcium in the cell sap, calcium oxalate crystals in the cells, stripping of the root cap and piliferous layer, and for transverse breaks in the cortex. Seeds were tested with picrolonic acid for the presence of calcium, and cultures were set up with isolated embryos.

TABLE I

Effect of Calcium on Symbiotic and Asymbiotic Seedlings

	Conditions of growth					
	With fungus		Without fungus		Embryo only	
	Nutrient plus Ca	Nutrient minus Ca	Nutrient plus Ca	Nutrient minus Ca	Nutrient plus Ca	Nutrient minus Ca
Excess Ca in cell sap	–	–	+	±	+	–
Calcium oxalate crystals	+	±	–	–	–	–
Stripping	±	–	+	±	+	±
Breaks in cortex	–	–	+	–	+	–

+ Positive or present. – Negative or absent. ± Trace.

The results, shown in Table I, demonstrate that in the absence of the fungus there is an excess of calcium in the cell sap, and that stripping of the cells and breaks in the cortex occur as a result, presumably, of the undue impregnation by calcium of the pectic wall of the cell. The slight deposition of crystals in those symbiotic plants grown in a solution without calcium is the outcome of the presence of calcium in the seed. When the fungus is present there is slight stripping of the piliferous layer during the early stages of germination, but once the fungus begins to ramify through the cells of the root this phenomenon ceases: there are no breaks in the cortex.

The fungus appears to play some part in stabilizing the calcium metabolism of the plant, owing to the deposition of calcium oxalate crystals and the consequent removal of excess calcium from the cell sap, thus preventing its combination in too great a quantity with the pectic substances in the wall.

DISCUSSION

The term 'cyclical symbiosis' first used by De Jongh lies at the core of this work on *Helianthemum chamaecistus.* All aspects of the problem are bound together in these two words. Is this fungus a cyclical one? Does it really pass from one generation to another through the seed, and is its presence of any value to the plant at one or at all stages in its life history?

When a fungus permeates through the tissues its presence is frequently deleterious to its host; if it is present in the seed it often parasitizes the young seedling at germination. Examination of the mature tissues of *H. chamaecistus* showed that the presence of the fungus was not harmful to the plant: it was more difficult to prove that it was beneficial. Germination and subsequent development of the seedling, however, is dependent on the presence of the fungus in the seed. Without it the plant ceases to grow beyond a certain stage, the plumule does not emerge, and the primary root does not branch. It seems therefore that during germination the presence of the fungus is essential. In orchids infection of the seedling takes place from the soil; here in *Helianthemum* the fungus is present in the seed and infection of the young seedling is thus assured. The fungus enters the seedling root and penetrates from here into the hypocotyl and plumule; it keeps pace with growth and differentiation at the apices and enters new tissues as they arise. The identity of the root fungus with that in the shoot is therefore seen very clearly in the seedling, though in the mature plant the connexion between the two is usually severed. The development of the fungus in the shoot was followed: it was seen in an active state in the young vascular parenchyma elements just behind the apical meristem and as new tissues were formed the fungal hyphae were seen to penetrate them. In this way the fungus gains access to the floral meristem and later the ovary wall. Its presence in the funicle of the seed was recorded in every specimen examined, and in no case was the gelatinous coat of the seed found to be devoid of a branching, septate mycelium. These facts are considered to be proof that the fungus of *Helianthemum chamaecistus* is a cyclical fungus, and that the host plant is a dual organism, harbouring a fungus in the seeds so that the association becomes hereditary.

The presence of the fungus has been shown to be essential during early stages of growth. Is it essential in the mature plant too, or is it only tolerated here because its presence in the seed is essential and because this is the only way for its presence in the seed to be assured? Examination of the root throughout the year suggests that its presence is beneficial to the plant. In spring and summer it ramifies through the cells, and enters new roots as or soon after they arise. In autumn, however, the hyphae are seen as swollen empty structures in the cells. It is reasonable to suppose that the plant has utilized the fungal material in the cells by means of digestive enzymes. In the stem there are no signs of digestive processes; here the fungus seems to be tolerated so that it may gain access to the seed.

The higher plant thus benefits in two ways at least from the presence of the fungus. Successful germination only occurs when it is present, and the endophyte in the root is digested and used as a source of nutriment. The fungus must obtain soluble food from the plant, for it has no contact with the soil except for one short period when it passes through an ectotrophic phase. It also solves, as a result of this association, the problem of the perpetuation of the species. It no longer needs to form reproductive organs; it reproduces its own species by the simple means of entering every seed in the ovary. Successful germination of the seed results in the renewal of growth of the fungus which remains dormant in the seed. When germination fails, then the fungus dies. It never lives outside the plant and it is doubtful whether it is capable of doing so. The metabolic and reproductive cycles of the host and the endophyte are both intimately bound one with the other, and we appear to have an association which is symbiotic in the true sense of the word.

SUMMARY

1. The presence of a branching septate fungal mycelium has been recorded in the outer coat of the seeds of members of the family Cistaceae, and its life-cycle elucidated in *Helianthemum chamaecistus*.

2. The fungus enters the seedling from the seed and spreads throughout the plant, keeping pace with the differentiation of tissues at the apices, and entering new tissues as they arise.

3. It enters the ovary, and passes up the funicle into the gelatinous coat (the outer integument) of the seed. It is prevented from entering the seed proper by a plug of tannin-filled cells at the base of the ovule and by an unbroken layer of similar cells inside the testa.

4. The embryo and endosperm are the only parts of the plant which do not contain the fungus.

5. The fungus passes from generation to generation through the seed. The relationship between fungus and plant is a hereditary one.

6. Two types of roots occur: normal long roots and *tuberous roots*. These latter are infected by the fungus while inside the parent cortex. This results in the swelling of the cells and in the loss of the meristematic activity.

7. In autumn the fungus in the older roots undergoes digestion, the hyphae swell, break up into small pieces, and lose their contents. It is not digested in the new long roots, and thus serves as a source of infection in the following spring. The intercellular mycelium of the tuberous root grows out to the surface in the autumn, forming a mat of ectotrophic growth.

8. Growth of the seedling ceases at a certain point in the absence of the fungus: the plumule does not emerge and no branch roots are formed.

9. It is suggested that one of the functions of the fungus is to provide thiamin to the seedling, thus relieving an avitaminosis of the plant. The fungus does not fix atmospheric nitrogen.

10. The term *cyclical symbiosis* has been used to describe the association between the seed-borne fungus and the higher plant.

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In a further paper it is hoped to give an account of experiments in which successful asymbiotic germination was brought about, and details of attempts to isolate the fungus.

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EXPLANATION OF PLATE

Illustrating J. G. Boursnell's article 'The Symbiotic Seed-borne Fungus in the Cistaceae'.

Fig. 1. Fungal mycelium in the cells of the gelatinous coat of the seed of *Helianthemum chamaecistus*. (× 100.)

Fig. 2. Hyphal coil in cortical cell of the root of *Helianthemum chamaecistus*. (× 650.) *h*. normal hypha; *h.c.* hyphal coil.

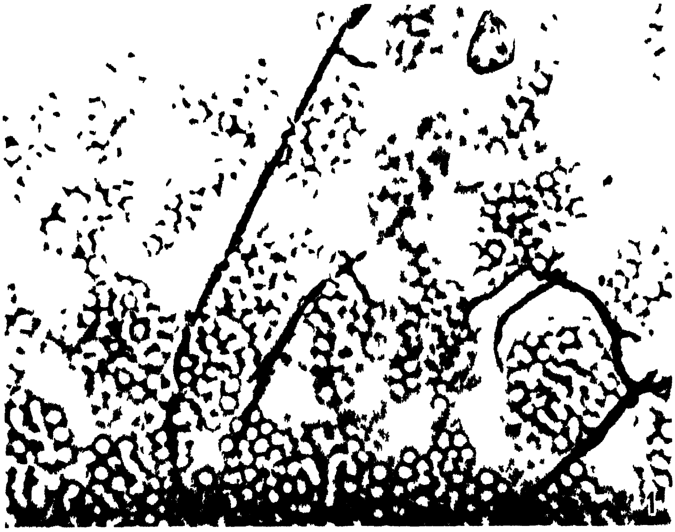

Fig. 1. Fungal mycelium in the cells of the gelatinous coat of the seed. ×100

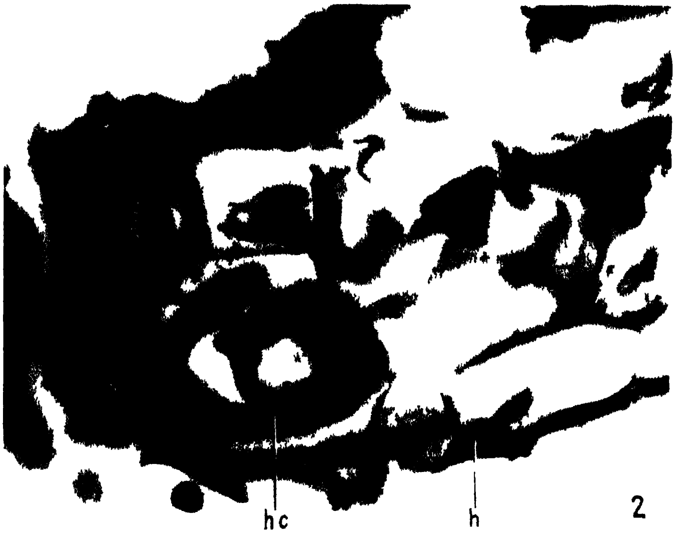

Fig. 2. Hyphal coil in cortical cell of root. ×650

J. G. BOURSNELL

Symbiosis of Leguminous Plants and Nodule Bacteria

III. Observations on the Growth of Soya Bean in Water Culture

BY

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INTRODUCTION

ALTHOUGH the technique of water culture has been employed extensively with normal autotrophic plants, it has been utilized relatively infrequently with nodule-bearing leguminous plants, and when, in connexion with experiments already described (Bond, 1941), it became necessary to grow nodulated soya-bean plants in water culture, it was found that relatively little guidance as to procedure could be obtained in the literature. As the result of experience then gained, some aspects have been investigated further, and in the present paper experiments extending over several years are described in which particular attention has been paid to the effects of aeration and the oxygen factor in nodulated soya-bean plants growing in water culture. Data are also presented on growth in different culture solutions and on the pH relation. The inclusion in some of the experiments of plants free of nodules and supplied with combined nitrogen, in addition to nodulated plants in nitrogen-free solution, assists in the determination of how far any effects observed are peculiar to plants associated with, and dependent on, nitrogen-fixing bacteria.

A review of the literature on the effect of forced aeration of water cultures of non-leguminous plants is included in the paper by Erickson (1946). In many instances the aeration of water cultures of ordinary type (i.e. with the cover not air-tight and with an air-space above the solution) has been found to benefit plant growth; in other cases no benefit has followed. Such discrepancies doubtless reflect the varying oxygen requirements of different species and may also have arisen from variations in experimental detail, such as the shape of the culture vessel. Several previous investigations on the effect of aeration in which soya-bean plants (non-nodulated and supplied with combined nitrogen) have been used are of special interest and will be considered in the Discussion.

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The effect of forced aeration of water cultures of nodulated leguminous plants was investigated in the experiments of Virtanen and von Hausen (1936) in which peas were grown aseptically in 3-litre Woulff's bottles containing nitrogen-free Hiltner's solution. In non-aerated cultures abundant nodules developed but remained relatively small; the average fixation of nitrogen was 10 mg. per plant, with a dry weight of 1·03 g. With aeration nodules were larger, fixation was 73 mg. per plant, and dry weight 2·64 g. The plants in a single culture of uninoculated peas supplied with excess of ammonium sulphate, without aeration, were considered to show good growth, gave a dry weight of 1·16 g. and a nitrogen content of 80 mg. per plant. The results of the experiment were ascribed to the effect of the oxygen content of the medium, which was considered to be adequate without aeration for root but not for nodule development and function, the latter having, it was concluded, a relatively high oxygen requirement.

Evidence on the last question is also provided by the investigations of Wilson and Fred (1937), in which clover plants were grown in sand culture totally enclosed in large bottles, the atmosphere within these being adjusted to contain different partial pressures of oxygen. At the lowest partial pressure the proportion of oxygen present was only $\frac{1}{16}$th of that in normal air. The response of nodulated plants in nitrogen-free medium was similar to that of non-nodulated plants receiving ammonium nitrate. Hence there was no evidence of a special requirement of oxygen by any part of the nodulated plant, a finding which is not readily reconciled with that of Virtanen and von Hausen (above).

Direct observations are available on the oxygen uptake of nodules and of their respiratory activity as compared with roots, but these observations also are discrepant. Allison (F. E.) et al. (1940) presented extensive observations by manometric methods on the oxygen uptake and carbon dioxide production of excised nodules and root fragments supplied with glucose. The rate of respiration of nodules was not appreciably higher than that of root tissues. Demolon and Dunez (1943) in observations on pulped nodule and root tissues found that the rates of carbon dioxide production were similar. Asprey and Bond (1941), on the other hand, in manometric observations on excised nodules and roots of soya-bean grown in water culture, found the oxygen intake per unit dry weight of nodules to be 2–3 times that of roots, with comparable differences in respect of carbon dioxide evolution.[1] Bond (1941), in observations on intact plants of soya bean in water culture, concluded that the evolution of respiratory carbon dioxide per unit dry weight of nodule tissues was approximately 3 times that from root tissues. It may be pointed out that the observations of high intake of oxygen in the presence of excess of that gas does not necessarily mean that nodules will be unable to function efficiently under reduced oxygen tension. Anaerobic mechanisms may be present which come into play under such a condition.

[1] For a description of the respirometer used in these observations see Asprey, G. F., 1948: An Apparatus for following the Course of Gaseous Exchange by Living Tissue. Proc. Roy. Soc. Edin., B. lxiii, 163–76.

METHODS

The Manchu variety of soya bean has been used in these experiments, seed being kindly supplied by Dr. W. J. Morse, United States Bureau of Plant Industry. Seeds of closely similar weight, after surface-sterilization, were sown in troughs of sand. At the stage when the plumule was unfolding, usually after about 12 days' growth, uniform seedlings were transplanted to static, non-flowing water culture in wide-mouthed glass jars of capacity 2½ litres. The jars were 20 cm. in height, 9 cm. and 15 cm. in diameter at the mouth and below the shoulder respectively, and were equipped with loose-fitting waxed teak tops.

The culture solutions used in the experiments have been based on a nitrogen-free form of Crone's solution. Bryan (1922) reported that the Crone medium (soluble portion only) was satisfactory for the cultivation of nodulated soya bean. Various formulae for the Crone medium may be found in the literature. The original formula devised by von der Crone (1902), modified here to a nitrogen-free form, is shown in the accompanying table, together with other formulae relevant to the present experiments. Quantities are g. per litre.

	Original.	Used by Bryan.	Formula A.	Formula B.
KCl	0·75*	0·75	0·75	0·75
$CaSO_4 \cdot 2H_2O$	0·5	0·19	0·19	0·25
$MgSO_4 \cdot 7H_2O$	0·5	0·19	0·19	0·25
$Ca_3(PO_4)_2$	0·25	0·19	0·19	0·25
$Fe_3(PO_4)_2 \cdot 8H_2O$	0·25	0·19†	0·19	0·25

* Replacing 1 g. KNO_3 of Crone's formula.

† $FePO_4$ was used instead of the ferrous salt.

Of the experiments described in the present paper, in those of 1946 formula A was used, while in 1947–8 formula B was substituted. It was believed at the time that formula B (Miller, 1938, p. 242) was the true Crone formula, but subsequent reference to the original paper of Crone (loc. cit.) showed that this was not the case. Tap-water (very low in available nitrogen) was employed in the preparation of the solutions.

Prior to use the solution was autoclaved at 20-lb. pressure for 15 minutes, and then stored in glass tanks until required. The pH of the autoclaved solution was 6·2. In some cases only the clear supernatant solution, obtained after the autoclaved mixture had stood in the storage tanks for 24 hours or more, was used, separation being effected by siphoning. In other experiments the medium was thoroughly stirred before use so that an appropriate amount of sediment was included, giving what will be termed the 'complete' solution. In a few cases what will be termed an 'intermediate' solution has been employed, and was prepared by adding 125 c.c. of 'complete' solution to each culture jar and then making up to 2½ litre with 'supernatant' solution. Minor elements (boron, manganese, zinc, and copper) were supplied to all jars in the form and amounts recommended by Loomis and Shull (1937, p. 60).

At weekly or fortnightly intervals (as indicated later) the contents of the culture jars were replaced with fresh solution. Sterile tap-water was added daily to maintain the solution 1–2 cm. below the teak tops.

Immediately after the young plants had been transferred to water culture, certain jars were inoculated with an effective strain of the soya-bean nodule organism (Wisconsin strain No. 9 in the first experiment of 1940, No. 505 in subsequent experiments). The remaining jars (apart from controls) were supplied with combined nitrogen in the form of sodium nitrate, which was added at frequent intervals in amounts estimated to be in excess of the requirements of the plants.

For aeration use has been made of two sources of air. In some cases an electric air-pump, compressing greenhouse air, was employed, the air being supplied for 16 hours out of each 24 hours, during hours of daylight. Alternatively air from commercial cylinders[1] was used, and in this case was supplied continuously to the plants. Dispersal of the air stream was obtained by fitting to the lead-in tube in each jar a cubical aerator of porous porcelain (2·5 cm. edge) of a type used for aquaria. The rate of flow was in general of the order of 1 litre per hour, but was varied somewhat according to the stage of development of the plants and the results of oxygen determinations (below), the object being to maintain oxygen tensions in the culture solution as near as possible to equilibrium values for water freely exposed to air.

Measurements of pH were usually made by glass electrode on samples withdrawn from midway down the jars. In certain experiments steps were taken to stabilize pH, which, as shown later, otherwise falls below the original value of 6·2 in jars of nodulated plants in Crone's solution. The procedure was to add daily to each jar sufficient N/10 NaOH to bring the pH to approximately 6·5.

Determinations of dissolved oxygen in the culture solution were made by the Winkler method, using the 10-c.c. syringe-pipette described by Whitney (1938), with precautions to avoid the introduction of manganous chloride into the culture jars. By fitting a cannula of suitable length, samples were withdrawn from levels midway down or near the base of the jars (9 cm. and 15 cm. respectively from the surface of the solution). All samples were taken during hours of daylight, and not less than 2 days after the weekly or fortnightly renewal of the solution. In order to facilitate comparison of oxygen data, results have been expressed as a percentage of the oxygen content of water in equilibrium with the atmosphere at the temperature and pressure prevailing at the time of the determinations, the data of Winkler (1889) being utilized for these calculations.

In the experiments, which were carried out in unheated greenhouses, cultures receiving the various treatments were, so far as was practicable, arranged in random manner on the benches, and positions were changed at frequent intervals. The experiments were continued for 13–16 weeks, by which time the plants were at the stage of forming young pods. Harvesting was then carried out, dry weights being obtained by heating the plant material at 95° C. and total nitrogen by Kjeldahl method.

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[1] It is understood from the British Oxygen Co. Ltd. that this air is pumped in a water-lubricated compressor.

EXPERIMENTAL RESULTS

1940 experiments. In two small-scale experiments the 'supernatant' Crone solution was employed, though separation from the sediment was probably less complete than in later experiments. The solution was renewed fortnightly and there was no adjustment of pH. The air-stream supplied to certain jars was provided by electric pump.

Results are presented in Tables I and II, and show that aeration increased nodulation, dry weight, and nitrogen fixation[1] in nodulated plants, while growth of combined nitrogen plants (Table I) was equally good with or without aeration. Determinations on samples of culture solution drawn from midway down the jars of the second experiment when the plants were 15 weeks old showed oxygen contents in aerated jars to be 90–95 per cent. and in non-aerated jars 60 per cent. of equilibrium figures.

TABLE I

Harvest Data for 1940 Experiment, Growth Period 15 May–9 August (data are per jar of 3 plants)

	Jar No.	No. of nodules.	Dry wt. of nodules (g.).	Dry wt. of roots (g.).	Dry wt. of tops (g.).	Total dry wt. of plants. (g.).	Total N content of plants (mg.).
			Nodulated Plants				
Non-aerated	1	221	1·25	0·90	5·08	7·23	155
	2	236	1·30	1·01	5·64	7·95	158
	3	122	0·71	0·70	3·76	5·17	93
Aerated	4	408	1·75	1·02	9·80	12·57	395
	5	491	1·81	0·98	9·75	12·54	418
			*Non-nodulated Plants**				
Non-aerated	1	—	—	5·25	22·74	27·99	403
	2	—	—	5·83	21·55	27·38	428
Aerated	3	—	—	5·64	24·58	30·22	432
	4	—	—	4·74	25·53	30·27	433
	5	—	—	4·17	19·55	23·72	418

* Supplied with a total of 500 mg. nitrate-nitrogen per jar.

1947 experiment. Both 'supernatant' and 'complete' Crone solutions were used with weekly renewal, with two sources of air. Daily adjustment of pH to 6·5 was applied to all jars of nodulated plants. Data obtained at harvest are given in Table III. With nodulated plants the 'complete' solution gave

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[1] Fixation of nitrogen per jar of 3 nodulated plants in the experiments described in this paper is obtained by deducting 20 mg. from the nitrogen contents shown in the tables. This deduction is based on the examination of control jars of non-nodulated plants not supplied with combined nitrogen. There are good reasons for assuming that there was no loss of fixed nitrogen by excretion in the present experiments (Bond, 1941, p. 321).

TABLE II

Harvest Data for 1940 Experiment, Growth Period 12 June–24 October, all Nodulated Plants (data are per jar of 3 plants)

	Jar No.	No. of nodules.	Dry wt. of nodules (g.).	Dry wt. of roots (g.).	Dry wt. of tops (g.).	Total dry wt. of plants (g.).	Total N content of plants. (mg.)
Non-aerated	1	114	0·58	0·97	4·70	6·25	153
	2	115	0·59	1·19	5·18	6·96	168
	3	133	0·36	0·84	4·92	6·12	145
	4	220	0·48	0·85	5·22	6·55	157
	Means	**146**	**0·50**	**0·96**	**5·01**	**6·47**	**156**
Aerated	5	259	0·52	1·32	9·03	10·87	297
	6	250	0·74	1·01	8·89	10·64	298
	7	360	0·59	1·08	7·11	8·78	242
	8	265	0·70	1·46	8·64	10·80	297
	Means	**284**	**0·64**	**1·22**	**8·42**	**10·27**	**284**

TABLE III

Harvest Data for 1947 Experiment, Growth Period 15 May–15 August (data are means per jar of 3 plants)

Nodulated Plants

Treatment.*	No. of replicate jars.	No. of nodules.	Dry wt. of nodules (g.).	Dry wt. of roots (g.).	Dry wt. of tops (g.).	Total dry wt. of plants (g.).	Total N content of plants (mg.).
S. N.A.	3	205	0·600	0·84	5·64	7·08 (6·6–7·8)†	227
S. A.	3	302	0·695	0·83	6·10	7·63 (6·9–8·1)	245
C. N.A.	3	406	1·362	1·03	13·22	15·61 (14·6–16·2)	495
C. A.	5	655	1·442	1·30	12·95	15·69 (14·7–17·7)	480
			Non-nodulated Plants‡				
S. N.A.	3	—	—	3·38	21·30	24·68 (23·7–25·4)	516
S. A.	6	—	—	3·94	24·91	28·85 (24·4–33·2)	511
C. N.A.	3	—	—	4·22	26·72	30·94 (28·6–32·7)	548
C. A.	3	—	—	3·21	20·00	23·21 (21·7–26·0)	523

* In this column S and C indicate 'supernatant' and 'complete' Crone solution respectively.
† Bracketed figures in this column show the range of variation in the replicate jars.
‡ Supplied with a total of 800 mg. N as sodium nitrate per jar.

growth which was superior in all respects to that obtained in the 'supernatant' solution. Non-nodulated plants receiving nitrate-nitrogen developed equally vigorously in both solutions. In jars containing these latter plants pH showed some fall from the original value of 6·2 during early stages of growth, particularly in the 'supernatant' solution, but later rose to values in the range of 6–7.

With both non-nodulated and nodulated plants the pH in aerated jars was usually higher than in non-aerated jars, the actual difference ranging from 0·1 to 1·0. With nodulated plants in both culture solutions the number of nodules was markedly increased by aeration,[1] without, however, a corresponding increase in nodule dry weight. Aeration had a clearly marked effect on the length and distribution of roots, those in aerated jars extending right to the base of the jar, while in the absence of aeration root and nodule development was chiefly in the upper part of the jar, but in terms of dry weight and nitrogen content of nodulated plants there was little effect. With non-nodulated plants aeration affected the length and distribution of the roots as above, but in respect of plant dry weight no distinct effect can be discerned, having regard to the variation between replicate jars.

The means of 20 oxygen determinations on aerated jars when the plants were 11–12 weeks old were 88 per cent. for the midway level and 93 per cent. for the base. Data for non-aerated jars are presented in Table IV. For nodulated jars the means of all the values are 31 and 21 per cent. for the midway and basal levels respectively, the corresponding means for non-nodulated plants being 58 and 32 per cent.

TABLE IV

*Oxygen Data for 1947 Experiment**

Days from commencement of experiment.	Temperature °C.	Sampling level.	Oxygen content of solution. Jars with nodulated plants.	Oxygen content of solution. Jars with combined N plants.
78	20	Midway	19	48
81	21	Midway	35	60
		Base	18	19
83	19	Midway	39	67
		Base	29	38
84	21	Midway	30	56
		Base	17	38

* All observations were on non-aerated jars with 'complete' solution. Oxygen values are expressed as percentages of equilibrium figures (see text), each value being the mean of observations on 2–3 jars.

1948 experiment. Here the effect of aeration from commercial cylinders was studied on nodulated plants receiving a wide range of cultural treatment (see Table V) in order to determine whether the favourable effect of aeration observed in 1940 could be reproduced under any of the treatments.

The data for non-aerated jars in Table V show that the 'supernatant' solution again proved markedly inferior to the 'complete' solution, while the 'intermediate' solution (with weekly renewal) gave somewhat better growth than the latter solution. In the 'complete' solution, particularly where there

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[1] Since there was little difference in the response to the two sources of air, the data are grouped together.

TABLE V

Harvest Data for 1948 Experiment, all Nodulated Plants, Growth Period 28 April–2 August (data are means per jar of 3 plants, with 3 replicate jars for each treatment)

Treatment.*	No. of nodules.	Dry wt. of nodules (g.).	Dry wt. of roots plus nodules (g.).	Dry wt. of tops (g.).	Total dry wt. of plants (g.).	Total N content of plants (mg.).
C. 7. N.A.	964	1·33	3·22	8·93	12·14 (10·7–13·0)†	347
C. 7. A.	1,400	1·38	3·48	8·66	12·14 (11·3–13·1)	299
C. 14. N.A.	—	—	3·74	8·83	12·57 (10·6–15·3)	327
C. 14. A.	910	1·29	3·39	8·54	11·93 (10·1–13·5)	255
C. pH 14. N.A.	1,060	1·32	3·21	9·97	13·18 (12·5–13·6)	349
C. pH 14. A.	2,165	1·41	3·87	9·53	13·40 (12·5–14·4)	284
Int. 7. N.A.	624	1·71	3·71	10·08	13·79 (12·3–14·8)	373
Int. 7. A.	795	1·05	2·20	6·11	8·31 (7·6–9·5)	239
Int. 14 N.A.	530	1·33	3·13	7·83	10·96 (9·4–11·9)	284
Int. 14. A.	440	1·23	2·65	6·78	9·43 (8·5–10·4)	284
S. 14. N.A.	312	0·83	2·04	4·76	6·80 (6·9–7·3)	—
S. 14. A.	150	0·61	2·03	4·65	6·68 (4·3–8·9)	—

* In this column C., Int., and S. indicate 'complete', 'intermediate', and 'supernatant' Crone solution respectively. The figures 7 and 14 indicate weekly or fortnightly renewal of solution. N.A. and A. signify non-aerated and aerated jars respectively, while 'pH' indicates jars in which pH was restored each day to 6·5.

† Bracketed figures in this column show the range of variation in the replicate jars.

was pH adjustment, aeration was accompanied by a marked increase in the number of nodules, though without any change in nodule or total dry weight, while the nitrogen fixed tended to be lower. In the 'intermediate' solution (particularly with weekly renewal) nodule, root, and top dry weights decreased in the presence of aeration. The non-aerated plants in this solution showed bursting of the outer layers of the tap root and the development of fistular cortex; the nodules on these plants included some relatively very large ones. In the 'supernatant' solution aeration reduced the number of nodules, but in other respects the plants were too variable to permit of comparisons; non-aerated roots showed abnormalities as above. In all solutions aeration again led to a denser growth of roots and more abundant nodules in the lower part of the jars. Observations on plants from the 'complete' solution showed that in non-aerated jars 87 per cent. of the total number of nodules occurred on the upper 10-cm. zone of the roots, while in aerated jars only 33 per cent. were located in that zone, the rest being at lower levels.

Comment on the pH and oxygen data, which are summarized in Tables VI and VII respectively, will be reserved to the Discussion.

TABLE VI

*pH Data for 1948 Experiment (all nodulated plants)**

Type of jar.‡	Days from commencement of experiment. 29.	36.	43.	50.	57.	64.	71.	80.	85.
Cultures with weekly renewal of solution†									
C. N.A.	6·29	6·10	6·18	5·96	6·04	5·87	5·67	5·28	5·50
C. A.	6·23	6·27	6·23	6·27	6·20	6·12	5·86	5·24	5·50
Int. N.A.	5·78	5·44	5·80	5·52	5·41	4·81	4·39	3·49	3·72
Int. A.	—	5·30	5·89	5·49	5·66	6·00	5·69	4·43	4·49
Cultures with fortnightly renewal of solution§									
C. N.A.	5·45		5·89		5·74		5·25		4·76
C. A.	5·33		5·70		5·84		4·97		4·79
Int. N.A.	4.16		4·71		4·52		3·68		3·48
Int. A.	4·23		4·77		4·31		4·18		3·61
S. N.A.	3·82		4·19		4·28		3·91		3·59
S. A.	4·03		4·29		4·25		4·25		4·00

* Each value is a mean for 2–3 jars.

† pH data shown were recorded 3 days after a renewal of solution.

‡ C., Int., and S. signify 'complete', 'intermediate', and 'supernatant' solutions respectively. N.A. and A. signify non-aerated and aerated respectively.

§ pH data shown were recorded 10 days after a renewal of solution.

TABLE VII

*Oxygen Data for 1948 Experiment (all nodulated plants)**

Days from commencement of experiment.	Temperature ° C.	Sampling level.	Oxygen content of solution. Non-aerated jars.	Aerated jars.
30	16	Midway	67	95
		Base	65	94
37	17	Midway	68	96
		Base	66	94
44	20	Midway	40	96
		Base	42	89
63	22	Midway	15	74
		Base	1	64
75	14	Midway	44	92
		Base	43	90
83	21	Midway	31	77
		Base	21	70

* All observations were on jars with 'complete' solution. Oxygen values are expressed as percentages of equilibrium figures (see text), each value being the mean of observations on 2–5 jars.

DISCUSSION

1. *Growth in different culture solutions*

In the above experiments the following versions of Crone's solution have been employed (see p. 247 for explanation of terms):

(*a*) 'Complete' Crone,
(*b*) as (*a*) but with daily adjustment to pH 6·5,
(*c*) 'Intermediate' Crone,
(*d*) 'Supernatant' Crone,
(*e*) as (*d*) but with daily adjustment to pH 6·5.

Considering nodulated plants first (non-aerated jars), solutions (*a*) and (*b*) both gave plants of healthy appearance and satisfactory fixation of nitrogen, the dry weight of plants in solution (*b*) being slightly superior (1948 experiment). Solution (*c*) in 1948 gave nodulated plants of slightly greater dry weight and nitrogen fixation than solutions (*a*) or (*b*), other features being that nodules were less frequent but of relatively large size, while the roots showed some abnormalities (p. 252). The generally favourable nature of this solution was a matter for surprise, since the addition of insoluble sediment was only a small one, and also the solution is of low buffering power, leading to the prevalence of relatively low pH. According to Bryan (1922) a pH of 5 or less is harmful to nodulated soya-bean plants if applied from the commencement of growth. As shown in Table VI, in jars receiving weekly renewal pH in solution (*c*) remained for the most part in the range 5–6 during the early weeks of growth in which most of the nodules were initiated. Subsequently, over the period in which most of the growth and fixation of nitrogen took place, pH was for a considerable part of the time below 4·5 and even below 4·0. These observations agree with those of Jensen (1943), who reported for *Medicago* and *Trifolium* that though the number of nodules formed is highest at a reaction near to neutrality, nodules once formed may continue to fix nitrogen at pH which is so low as to prohibit new nodule formation.

The experiments of 1947–8 with solutions (*d*) and (*e*) show that the soluble portion alone of the Crone medium does not support healthy growth of nodulated soya-bean plants in experiments of long duration.[1] Solution (*d*) is characterized by unstable pH in contact with nodulated plants, so that values in the region of pH 4 prevailed for a considerable part of the experiment (Table VI), but the fact that (*e*) in 1947 gave no better results suggests that pH is not mainly responsible. It is likely that the solution will be deficient in phosphorus, calcium, and iron on the basis of commonly accepted minimal requirements for culture solutions, while at the same time there is a large excess of potassium and chloride. Comparison of growth in solutions (*d*) and (*e*) with that in the solutions containing insoluble constituents indicates that the sediment is definitely of value to the plants (Marais, 1922; Miller, 1938, p. 244).

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[1] It is concluded that in the 1940 experiments, in which good growth was obtained in what was nominally 'supernatant' solution, sufficient sediment was included to prevent the deficiencies which arise when the solution is completely free of sediment.

Only solutions (*a*) and (*d*) have been employed with non-nodulated plants supplied with nitrate. In the direct comparison provided by the 1947 experiment, growth in both solutions was strong, that in (*d*) being not definitely inferior to growth in (*a*); pH changes were relatively slight with both solutions. Thus we have a solution which fails to support good growth of nodulated plants but which is satisfactory with non-nodulated plants receiving combined nitrogen, suggesting that the mineral requirements of the two types of plant differ. The scanty information on this question has been reviewed by Wilson (1940).

2. *Further analysis of pH drifts*

The main findings are: (1) the pH of solution in contact with nodulated plants falls with some rapidity, varying with the different versions of the Crone solution and with the stage of plant development; (2) with nodule-free plants supplied with nitrate there is no corresponding fall, and in later stages pH of the solution increases after contact with the roots of the plants; (3) with both types of plant pH in aerated jars is usually, though not always, higher than in non-aerated jars.

As regards (1) and (2) the possibility arose that the tendency for a depression of pH exists also with non-nodulated plants, but that it is counterbalanced by the well-known opposite tendency for the presence of nitrate as the sole source of nitrogen to lead to an increase in pH. That this is actually the case is indicated by tests carried out in 1948 on non-nodulated plants normally supplied with nitrate, specially grown for the purpose. In the jars of such plants, temporarily deprived of nitrate, a fall in pH from 6·2 to 4·5 was shown in 4 days, while in corresponding jars with nitrate present pH remained in the range 5·9–6·1.

This diminution in pH shown by Crone's solution in contact with nodulated and non-nodulated plants of soya-bean is no doubt due in part to accumulation of carbon dioxide, but the main factor is presumably the unequal absorption of the ions of the salts of the nutrient solution.

3. *Effect of aeration. Oxygen relation*

Aeration may tend to change conditions in the culture jars by preventing the depletion of oxygen in the solution, and also that of nitrogen where there is any utilization of that gas, as in fixation. The removal of respiratory carbon dioxide will be accelerated, which may in turn result in pH in aerated jars being somewhat higher than in those without aeration. Such differences in pH tended to exist in the present experiments, but for the most part were relatively small. In addition the air-stream will have stirring effects, which might, for example, result in the nodule bacteria being spread more effectively over the root system, giving greater infection, though this is not necessarily beneficial to the plants.

It will be assumed that, of the above, the oxygen factor is likely to be of chief importance. It is then necessary to consider the extent of the differences in dissolved oxygen between aerated and non-aerated jars with the arrangement employed in the present experiments, which allowed fairly free access of atmospheric oxygen to the surface of the culture solution in non-aerated jars. The data presented show that during the first half of the growth period oxygen tension of 99–100 per cent. of equilibrium values were maintained in aerated jars, tensions recorded later being mostly upwards of 75 per cent. of equilibrium, although some lower values were obtained. With non-aerated jars of nodulated plants, values in the region of 65 per cent. were obtained midway down the jars with plants 4–5 weeks old, after which tensions fell to somewhere in the range of 15–45 per cent. of equilibrium. With non-aerated jars of combined nitrogen plants the reduction in oxygen tensions was less marked. It was clear that oxygen content was closely dependent on temperature, being much lower on relatively warm days, no doubt as a result of the greater effect of increased temperature on root respiration than on the rate of diffusion of fresh oxygen into the solution. Oxygen content at the base of the non-aerated jars was usually lower than that at the midway level, and values at or near to zero were sometimes obtained for the former region (see also later).

It is clear from the above that the use of unsealed cultures and the method of depending on the activity of the plants to produce a deficiency of oxygen result in poor control over oxygen content. The extent of the oxygen deficiency is not steady during the experiment, but increases as the plants become larger and to a different degree in jars containing nodulated and non-nodulated plants respectively. No determinations were carried out on the solution above the midway level, but it may be assumed that oxygen tensions there in non-aerated jars were higher than those at midway, and that at least in the earlier stages of the experiments little difference existed at these higher levels between aerated and non-aerated jars.

The differences in growth and activity actually observed between plants of aerated and non-aerated jars in these experiments have been indicated in the previous section and, for nodulated plants, are obviously too discrepant to attempt at this stage any unified treatment or final conclusions. The following observations may, however, be made.

1. In the 1940 experiments aeration resulted in all-round benefit to nodulated plants, i.e. the result resembled that obtained by Virtanen and von Hausen (1936; see Introduction to the present paper) for peas with an essentially similar experimental arrangement. In later trials with varied experimental procedure no definite benefit from aeration to nodulated plants was observed and in some respects it was detrimental. An explanation of these divergent results cannot be offered at present. The nature of the result seems to be affected by the type of culture solution employed, and possibly by climatic and other factors not at present appreciated.

2. The sparsity of roots and nodules in the lower regions of non-aerated

jars suggests that oxygen tension there was limiting, but the ability of the roots and nodules to develop relatively freely in the higher oxygen tensions prevailing in the upper parts of the jars, both with nodulated and non-nodulated plants, means that a clear demonstration of the relative importance of oxygen to the two types of plants is not necessarily given by the experimental arrangement used in the present work, since taking the jar as a whole critical oxygen tensions may not be attained. The possibility of variation in result from one experiment to another exists, since under conditions conducive to rapid growth and exhaustion of oxygen, limiting values of the latter may be approached to a greater extent than when growth is slower. Experiments with an improved arrangement, resembling that employed by Gilbert and Shive (1942) and giving closer control over oxygen tension, are in progress.

3. Bearing in mind the marked depletion of oxygen recorded for the midway level in the non-aerated culture jars during the later periods of the 1947/8 experiments, and that an appreciable proportion of the roots and nodules occur in this region, the data presented suggest that roots (of both nodulated and non-nodulated plants) and nodules are able to function without serious diminution of efficiency under appreciably reduced oxygen tension, since dry weights and nitrogen fixation for non-aerated plants were not lower than for aerated plants.

4. It is felt that the increase in the number of nodules observed in most instances as a result of aeration may be due to a stirring effect, since during the earlier stages of the experiments when nodule initiation was most active the differences in oxygen tension between aerated and non-aerated jars were not marked.

5. The results obtained with combined-nitrogen plants may be compared with those of Gilbert and Shive (1942), who, using sealed constant-flow cultures of non-nodulated plants of soya-bean supplied with nitrate-nitrogen, studied the effects of passing nitrogen/oxygen gas mixtures containing respectively 40, 20, 10, and 0 per cent. oxygen for a period of 23 days. Although symptoms ascribed to oxygen toxicity were observed at 40 and 20 per cent., being apparently confined to the tops, differences in dry weight were not very marked except at zero oxygen, and taking dry weight at 40 per cent. oxygen as 100, the relative figures for the progressively lower proportions of oxygen were 94, 95, and 79. In the present experiments oxygen tensions recorded midway down non-aerated jars of combined-nitrogen plants in later stages of growth were of the order of 50 per cent. of equilibrium, equal to that produced by passing a nitrogen/oxygen mixture containing 10 per cent. oxygen. Higher tensions no doubt prevailed at earlier stages, and also higher up the jars. That there should be little difference in growth between plants in such jars and those in jars receiving aeration is in general agreement with the results of Gilbert and Shive.

6. Allison and Shive (1923) examined the response to aeration of non-nodulated soya-bean plants in water culture (plus nitrate). In static cultures aeration resulted in greater root length and slightly increased root dry weight,

but the dry weight of the whole plants was slightly less than in non-aerated jars. With constant-flow cultures, on the other hand, aeration led to considerably increased dry weight. The authors suggested that some growth-retarding factor (the development of a somewhat unfavourable pH is mentioned) prevented a response to aeration in static cultures, in which growth, besides being smaller than with constant flow, was somewhat abnormal. In most of the experiments of the present paper healthy growth of plants was obtained, with no suggestion of abnormality or inhibition of growth in non-nodulated or nodulated plants. It may also be noted that increasing the frequency of renewal of the 'complete' solution from fortnightly to weekly in 1948 did not lead to greater growth.

7. Gilbert and Shive (1945) concluded that soya-bean and other plants (tomato, oat) have the ability to carry on a special type of anaerobic respiration in which the nitrate ion is used as a source of oxygen. Their results indicate that this nitrate respiration is at a maximum in zero oxygen, and that when the culture solution is maintained at 4 p.p.m. oxygen (equal to 50 per cent. of value for water in equilibrium with air) nitrate respiration falls to ⅛th of its maximum, presumably signifying that the plants are now making use chiefly of ordinary aerobic respiration. It thus appears that at the oxygen tensions prevailing in the non-aerated jars of combined-nitrogen plants in the present experiments, nitrate respiration was of little importance, and was unlikely to impart to those plants any special immunity to the effects of low oxygen tension not shared by the nodulated plants. In future experiments it will, however, be desirable to include plants supplied with ammonia-nitrogen in order to test the matter fully.

4. *Further observations on the rate of depletion of oxygen in non-aerated jars*

As noted in the 1947 experiment, oxygen tensions in non-aerated jars of nodulated plants were consistently below those in corresponding jars of combined-nitrogen plants. This suggests a greater rate of oxygen consumption by nodulated as compared with non-nodulated roots on a per plant basis, since opportunities for replenishment of oxygen by diffusion appear to have been at least as good in jars of nodulated plants as in those of non-nodulated plants.

In order to gain further data an experiment was carried out on nodulated plants and non-nodulated plants receiving nitrate (2 jars of each) from the 1947 experiment, when the plants were 12 weeks old. The jars were filled with fresh solution, and then by aeration the oxygen content was brought close to the equilibrium figure, when air-currents were stopped. A control jar with no plants was included. Changes in oxygen tension at midway and basal levels in the jars were followed at intervals during hours of daylight over a total period of 44 hours, during which day-time temperatures in the greenhouse ranged from 19° to 23° C. Table VIII shows that from the 5th hour onwards, oxygen tensions in the jars of nodulated plants were notably below those for the non-nodulated plants. By the 28th hour oxygen tension at the

TABLE VIII

Data of Experiment on Rate of Depletion of Oxygen

	Sampling level.	Oxygen content of solution*					
		Start.	1½ hours.†	5 hours.	20 hours.	28 hours.	44 hours.
Jars with nodulated plants‡	Midway	97	75	42	32	14	25
	Base	93	70	35	8	0	0
Jars with combined nitrogen-plants‡	Midway	96	80	51	64	47	48
	Base	94	69	33	45	21	23
Control jar, no plants	Midway	101	99	97	96	92	91
	Base	97	101	—	87	88	83

* Expressed as percentage of equilibrium figure (see text).
† Hours from start of experiment.
‡ Oxygen values for jars with plants are means of 2 jars.

base of the jars of nodulated plants was actually zero. The apparent fall in oxygen tension in the control jar is probably due to temperatures being somewhat lower in the later part of the experiment.[1] The mean dry weights for the plants used in this test were, at harvest shortly afterwards, as follows:

	Roots.	Nodules.	Total
Nodulated plants	1·04 g.	1·33 g.	2·37 g.
Non-nodulated plants plus nitrate .	4·41 g.	—	4·41 g.

These data support the conclusion that oxygen utilization by nodulated roots is considerably greater than by non-nodulated ones. Assuming respiratory quotients not greatly in excess of unity, the above conclusion is in keeping with that of previous experiments on plants grown under similar conditions (Bond, 1941; see Introduction to present paper) which indicated that the evolution of carbon dioxide per unit dry weight of soya-bean nodule tissues was considerably greater than for root tissues.

SUMMARY

1. Observations are presented on the growth in static, non-flowing water culture of nodulated plants of soya-bean in nitrogen-free solution, and of corresponding non-nodulated plants supplied with nitrate-nitrogen.

2. Satisfactory growth and fixation of nitrogen were obtained with nodulated plants in Crone's solution containing the full complement or a proportion of the insoluble constituents, but not in a medium comprising the soluble portion only. The insoluble constituents thus appear to be of value to the plants. Non-nodulated plants supplied with nitrate-nitrogen made satisfactory growth in the medium with soluble material only, suggesting a difference in mineral requirements between the two types of plant.

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[1] The method of expression of results of oxygen determinations adopted in this paper assumes instant re-establishment of equilibrium with the atmosphere when the temperature of the solution changes. Actually equilibrium will only be regained after an interval of time, and because of this the method of expression introduces small errors in respect of determinations made under conditions of fluctuating temperature.

3. The pH of Crone's solution in contact with nodulated roots fell from the initial value of 6·2 with some rapidity, varying with the different modifications of the solution that were employed. With nodule-free plants supplied with nitrate-nitrogen a corresponding fall in pH was not shown, but a fall did occur if such plants were temporarily deprived of nitrate.

4. Evidence was obtained that, after the period of maximum formation of nodules is passed, nodulated plants grow well at relatively low pH (3·5–5·0).

5. Well-marked differences in the oxygen content of the solutions midway down and at the base of the culture jars were shown between non-aerated and aerated jars, particularly in the later stages of growth. Differences were probably smaller in respect of the solution in the upper part of the jars, since the arrangement employed allowed fairly free access of atmospheric oxygen to the surface of the culture solution.

6. In the experiments of the first year aeration led to all-round benefit to nodulated plants, but in subsequent experiments under a wide range of cultural treatments, some of which appeared to reproduce closely the conditions of the first year, aeration led in most cases to greater root length and to more numerous nodules, but little or no benefit ensued in terms of dry weight or nitrogen fixation, which were sometimes better in non-aerated jars. Non-nodulated plants receiving nitrate-nitrogen grew equally vigorously with or without aeration in all instances.

7. No adequate explanation of these discrepancies with nodulated plants can be offered at present. In any case it is felt that for reliable data on the relative importance of oxygen to nodulated and non-nodulated roots it is essential to adopt a method giving closer control over the oxygen content of the culture solution. Experiments with an improved method are in progress.

8. The results do permit the conclusion that roots (of both nodulated and non-nodulated plants) and nodules are able to function efficiently at appreciably reduced oxygen tension.

9. In the absence of aeration the oxygen content of jars containing nodulated plants was usually less than that in jars with non-nodulated plants supplied with combined nitrogen, despite the greater mass of the roots of the latter plants. A more rapid intake of oxygen per unit dry weight of nodulated root is suggested, though it does not necessarily follow from this that such roots will be specially sensitive to reduced oxygen supply.

Dr. J. Boyes carried out preliminary experiments in connexion with the work described in this paper, and was responsible for several features of the experimental arrangement. A grant from the Agricultural Research Council has facilitated the investigation, in which technical assistance has been given by Miss M. P. McGonagle, and Messrs. T. P. Ferguson and J. C. Leitch. The author is indebted to Dr. S. Williams for his criticism of the text of the paper.

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Fungal Parasites of the Phytoplankton. I

(Studies on British Chytrids, X)

BY

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With one Plate and sixteen Figures in the Text

THE planktonic algae of four bodies of water in the English Lake District (i.e. Windermere, North and South Basins, Blelham Tarn, and Esthwaite Water) have been examined at weekly intervals from October 1946 to the time of writing, April 1949, for the presence of aquatic fungi. A few records of fungi prior to October 1946 are also included. The more important algae present in these lakes belong to the classes Chlorophyceae, Bacillariophyceae, Chrysophyceae, Xanthophyceae, Myxophyceae, Dinophyceae, and Cryptomonadineae. It is only on the latter class that no fungus, saprophytic or parasitic, has been observed. By far the most abundant fungi belong to the Uniflagellatae series of Phycomycetes, but a few Biflagellatae forms have been observed. Altogether thirty different organisms are now known, three being saprophytic and the rest parasitic. The parasitic fungi sometimes increase rapidly, reaching epidemic proportions, and in consequence a particular species of alga may have its numbers severely reduced. This has already been demonstrated (Canter and Lund, 1948) for the diatom *Asterionella formosa* Hass. which is parasitized by *Rhizophidium planktonicum* Canter. Other similar investigations are being carried out on *Fragilaria crotonensis* (A. M. Edw.) Kitton, *Melosira italica* (Ehrenb.) Kütz., *Sphaerocystis Schroeteri* Chod., *Gemellicystis neglecta* (Teiling) Skuja, and the many desmids which occur in the plankton.

This paper deals with the life-histories of a few of the more abundant chytrids. Many others have been noted, but the knowledge of their structure and life-cycles is not sufficient to warrant description at this stage.

In 1949 an investigation of phytoplankton parasites from seventeen other lakes in the English Lake District was started. At the time of writing this survey is only a few months old, but already many of the parasites common to Windermere, Esthwaite Water, and Blelham Tarn have been found, and records of their occurrence are given in the relevant tables.

I. *RHIZIDIUM WINDERMERENSE.* N. SP.

Gemellicystis is a colonial green alga surrounded by a wide mucilage sheath (Fig. 1, A) to which may be attached cells of *Chlamydomonas epiphytica*

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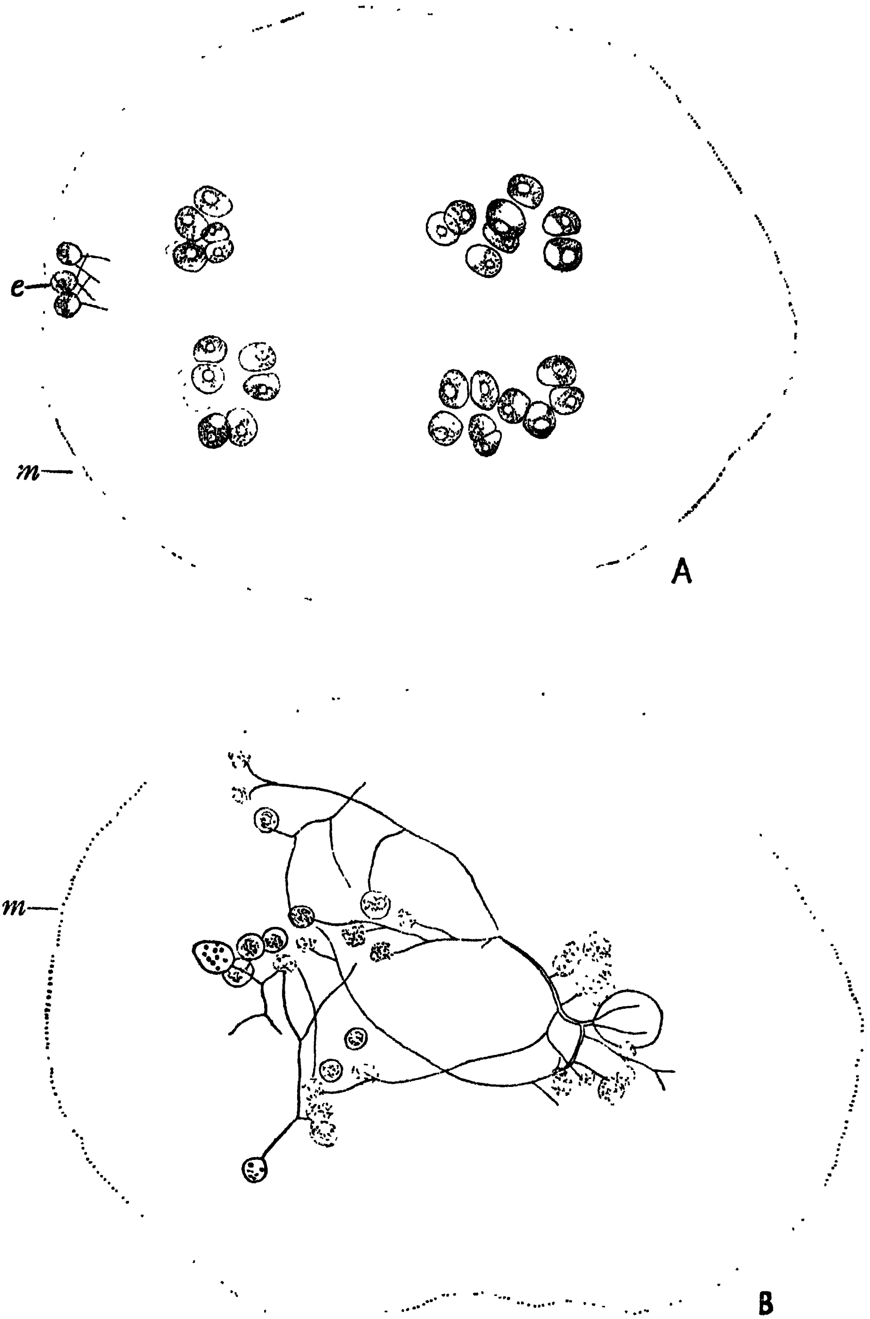

FIG. 1, A, B. *Rhizidium windermerense* n. sp. A, a healthy colony of *Gemellicystis neglecta* with three cells of the epiphyte *Chlamydomonas epiphytica*. Drawn by Dr. J. W. G. Lund. B, colony infected by *Rhizidium windermerense*. m, mucilage. (A, ×520; B, ×470.)

G. M. Smith (Fig. 1, A, *e*). The parasite is polyphagous and a single thallus may kill nearly all the cells in a host colony (Fig. 1, B). The content of the diseased cells shrinks and aggregates, and, in the advanced stages of infection, the cell walls tend to disintegrate.

TABLE I

Occurrence of R. windermerense *on* Gemellicystis neglecta (*Teiling*) *Skuja*

Lake.	1947.	1948.	1949.
Windermere: North Basin	June 15–Nov. 17	Aug. 30–Oct. 11	Apr. 19–May 9
Windermere: South Basin	June 11–Aug. 5, Oct. 14	Sept. 8–Oct. 26	Apr. 28–May 10
Blelham Tarn	Oct. 1–13	—	—
Esthwaite Water	June 13–July 10	July 12–23	—
Derwent Water	—	—	Feb. 10–Mar. 24, Apr. 7

The zoospore of *Rhizidium windermerense*, having penetrated for some distance into the mucilage surrounding the alga, gives rise to an unbranched or little-branched rhizoid which grows towards, and ultimately makes contact with, a host cell (Fig. 2, A). A lateral branch often develops close to the sporangial rudiment and grows in approximately the opposite direction to that of the original germ-tube so that the thallus may have a rhizoidal system, as shown in Fig. 2, I.

The rhizoids are long and taper towards their extremities. Fine branches pass to the individual host cells, but, owing to the dense content of the latter, the internal rhizoidal system, which presumably exists, has not been observed. Other fine branches penetrate through the mucilage (Fig. 4). The encysted zoospore enlarges directly into the sporangium. When young this is spherical (Fig. 2, B), but later a thin-walled apical papilla develops so that the mature sporangium is somewhat pear-shaped (Fig. 2, G). The sporangia vary from 8μ high by 6μ broad to 26μ high by 23μ broad and contain from 5 to 60 or more zoospores according to their size. Dehiscence has been observed only a few times, but there can be no doubt that this fungus is inoperculate. The zoospores emerge in a mass (Fig. 2, H) and are finally differentiated outside the sporangium. Each is spherical with a large oil globule and single posterior flagellum.

Towards the end of an epidemic caused by this chytrid sexually formed resting spores appear. Several may occur in a host colony, alone or mixed with asexual sporangia (Fig. 4).

Two spherical bodies (resembling encysted zoospores) which I have regarded as sexual thalli give rise to fine threads ('conjugation tubes'). These apparently meet at their tips. The content then passes from the sexual thalli along the conjugation tubes and a swelling is formed at their junction. This swelling (Fig. 3, A–D) is the incipient resting spore or zygote. At this stage

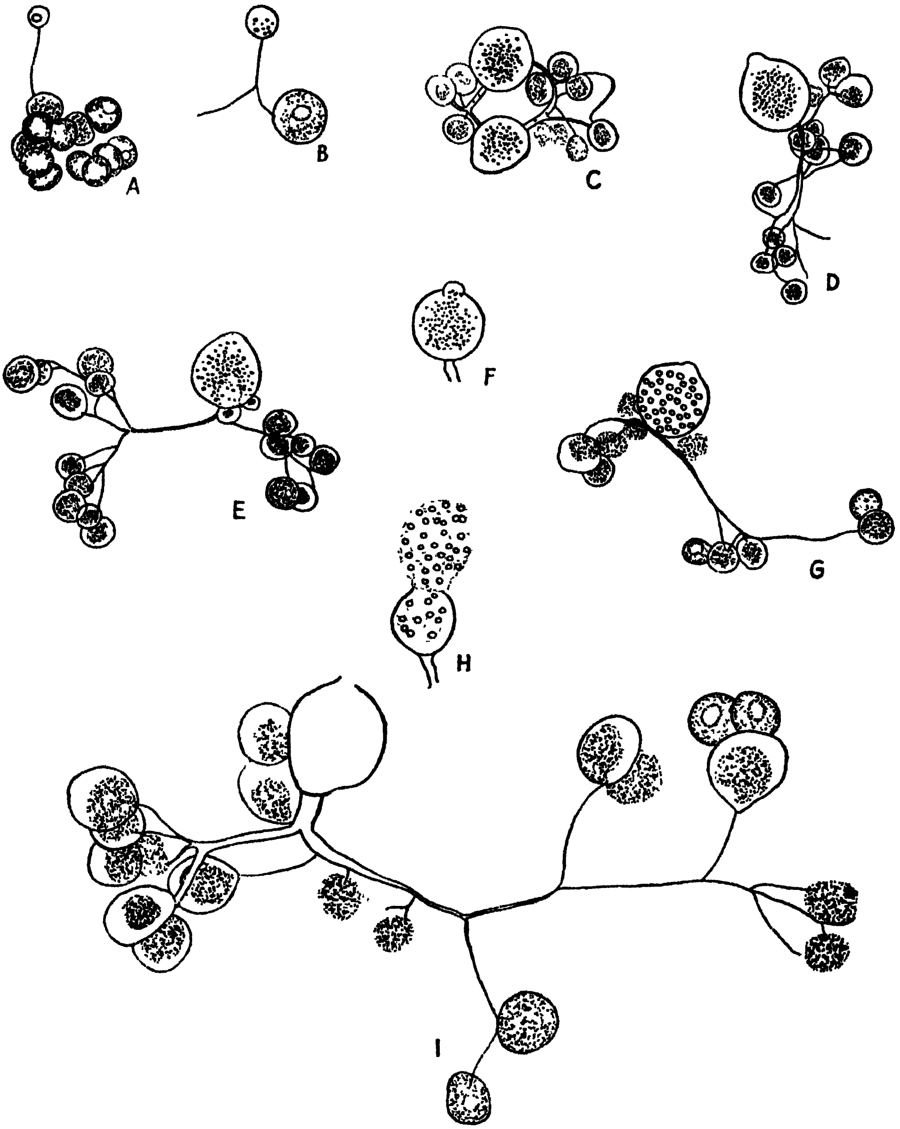

FIG. 2, A–I. *Rhizidium windermerense* n. sp. A, encysted zoospore with germ-tube. B–F, stages in the development of the sporangium, and growth of the rhizoidal system. G, mature, sporangium with dehiscence papilla. H, content emerging from the sporangium on dehiscence. I, empty sporangium with broad rhizoidal axes which taper towards their extremities. (A–F, H, ×500; B, I, ×850; G, ×512.)

the protoplasm is homogeneous and it is surrounded by a thin, colourless wall. There is apparently no constant difference in size between the fusing thalli. The empty sexual thalli vary from 2·6 to 5·9μ in diameter. At an early stage, perhaps even before the content passes out of the thalli, a few fine branches are formed on the conjugation tubes which may pass to a host cell

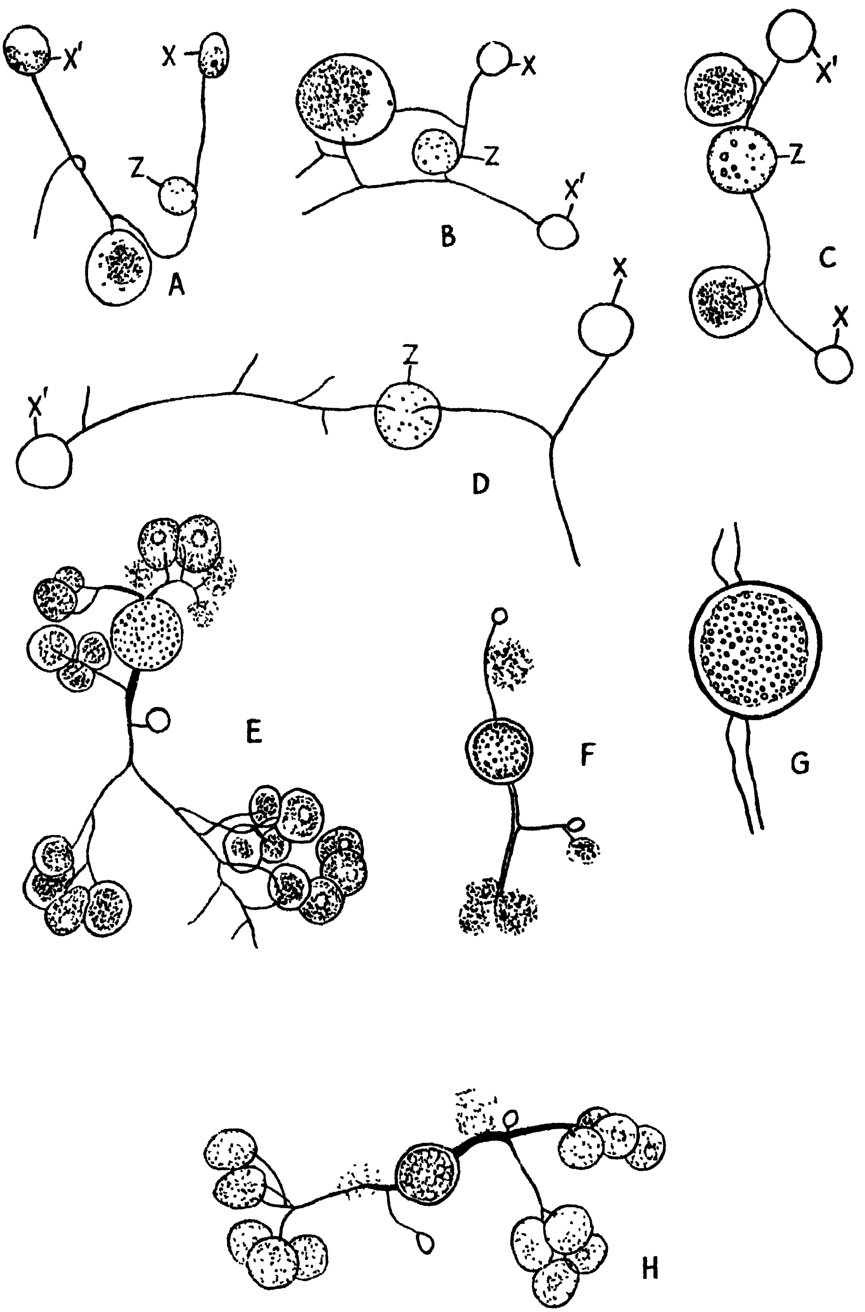

FIG. 3, A–H. *Rhizidium windermerense* n. sp. A, very early stage in fusion, content of associating gametes not yet wholly emptied. B–F, stages in growth of the resting spore; E, shows extensive rhizoidal system, and F the thickening of the zygospore wall. G, mature zygospore, with greatly thickened conjugation tubes, which now form a part of the rhizoidal system. H, mature resting spore and its rhizoidal system.

In figs. A–D: associating gametes, X, X^1; young resting spore, Z. (A–E, G, × 1130; F, × 670; H, × 800.)

or into the mucilage (Fig. 3, A, B). However, after protoplasmic fusion has taken place a large rhizoidal system is formed which resembles in its nature, and almost in extent, that of the sporangium (Fig. 3, E).

When mature the zygote is more or less spherical (14–17 μ). It has a thick, smooth wall which may become yellowish in colour, and the content consists of numerous small globules (Fig. 3, H).

In its polyphagus habit this fungus resembles species of *Rhizophlyctis* and *Polyphagus*. However, it differs from the latter in the zoospore enlarging directly into the sporangium. Although similar in development to species of *Rhizophlyctis* it again differs from this genus by virtue of its single-branched rhizoidal axis arising from the sporangium. This feature characterizes the genus *Rhizidium*, into which the writer places this fungus for the time being as a new species, *R. windermerense*. The method of formation of the resting spore is unlike any other chytrid, although it is not so far remote from that described by Sorokin (see Sparrow, 1943, p. 389) for *Zygochytrium aurantiacum*. However, it is presumably not monoecious, and although germination of the resting spore has not been observed, it seems unlikely that it produces a hypha-like structure as recorded for *Zygochytrium*.

In its sexual process it resembles *Polyphagus* and more especially *P. Euglenae* Nowak. sense nov. Bartsch, where the resting spore develops subterminally (see Bartsch, 1945, figs. 9, 12). However, in this genus the conjugating thalli have already grown to a considerable size before fusion takes place.

Rhizidium windermerense sp. nov.

Thalli monocentrici, eucarpici, polyphagi, sporangiis et rhizoideis principalibus et ramulis secundariis muniti. *Sporangium* inoperculatum piriforme, 8 μ altum × 6 μ latum–26 μ altum × 23 μ latum zoosporis 5–60, globosis, uniguttulatis, postice uniflagellatis. *Sporae* perdurantes (zygosporae) globosae vel subglobosae multiguttulatae, 10 μ diam., membrana crassa levi, ex conjunctione apicali rhizoideorum principalium thallorum duorum parvorum formatae. Hab.: In cellulis vivis *Gemellicystidiis neglectae* Windermere, Esthwaite Water, Blelham Tarn et Derwent Water, Anglia.

Rhizidium windermerense n. sp.

Thallus monocentric, eucarpic, polyphagous, consisting of a sporangium (the body of the encysted zoospore) and a single main rhizoidal axis which by the development of secondary branches loses its original taproot-like appearance. Sporangium inoperculate, pear-shaped, 8 μ high by 6 μ broad to 26 μ high by 23 μ broad, containing from 5 to over 60 zoospores. Zoospores spherical, uniguttulate and uniflagellate, emerging in an undifferentiated mass. Zygospore spherical to subspherical, 14–17 μ in diameter, wall thick, smooth, sometimes yellowish, content consisting of numerous small globules; formed after conjugation of the tips of the rhizoids from two thalli, the contents of both passing out to form a swelling, the incipient resting spore at their point of fusion. Rhizoidal system as in the sporangium developing after fusion of

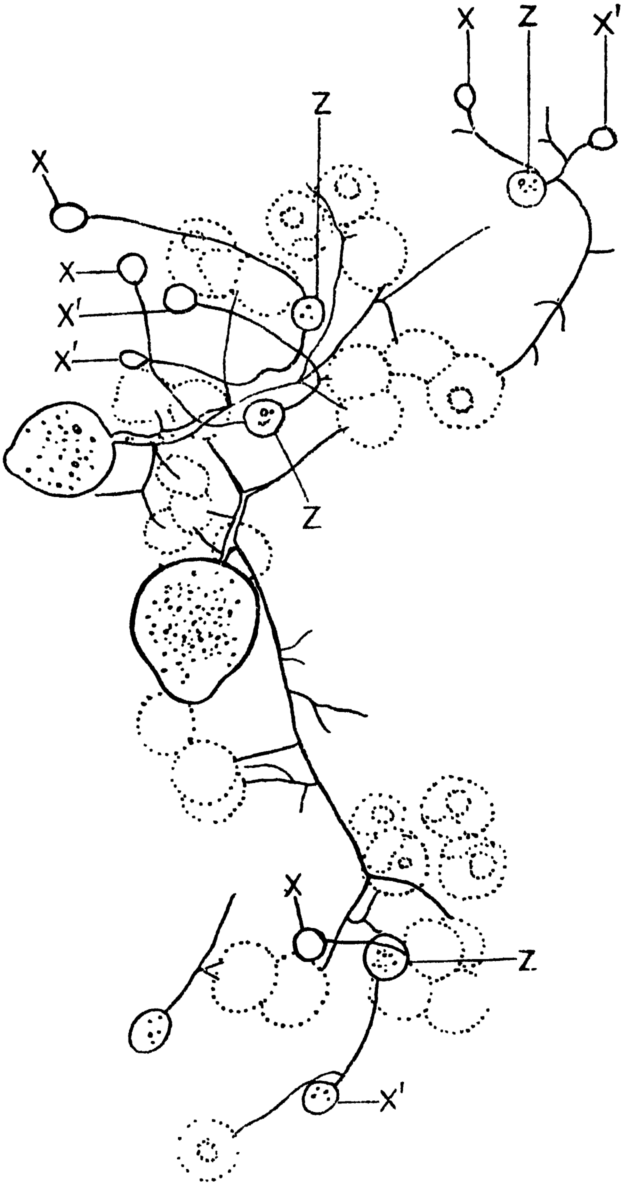

FIG. 4. *Rhizidium windermerense* n. sp. A *Gemellicystis* colony containing three zoosporangia and several very young zygospores. Associating gametes (x, x^1), zygospore (z). The contents of the host cells are omitted for simplicity. (× 1025.)

the sexual thalli. The latter remain as empty appendages (2·6–5·9μ diam.) on the rhizoidal system of the mature spore.

Parasitic on *Gemellicystis neglecta* (Teiling) Skuja from Windermere North and South Basins, Esthwaite Water, Blelham Tarn, and Derwent Water, in the English Lake District.

II. RHIZOPHIDIUM EPHIPPIUM N. SP.

TABLE II

Occurrence of R. ephippium *on* Stylosphaeridium stipitatum *Geitler*

Lake.	1948.
Windermere: North Basin .	July 19–Dec. 13
Windermere: South Basin .	July 14–Nov. 10
Esthwaite Water . .	Oct. 12

The zoospore, having passed through a part of the mucilage surrounding a *Coelosphaerium* colony, settles on the broad upper surface of a *Stylosphaeridium* cell[1] (Fig. 6, A). It swells and then enlarges laterally so that the young sporangium is rather bean-shaped and arches over the host cell (Fig. 6, F, G). As the sporangium matures, the sides become drawn out to form two lateral papillae (Fig. 6, K, L, M). The protoplasm never contains many oil globules

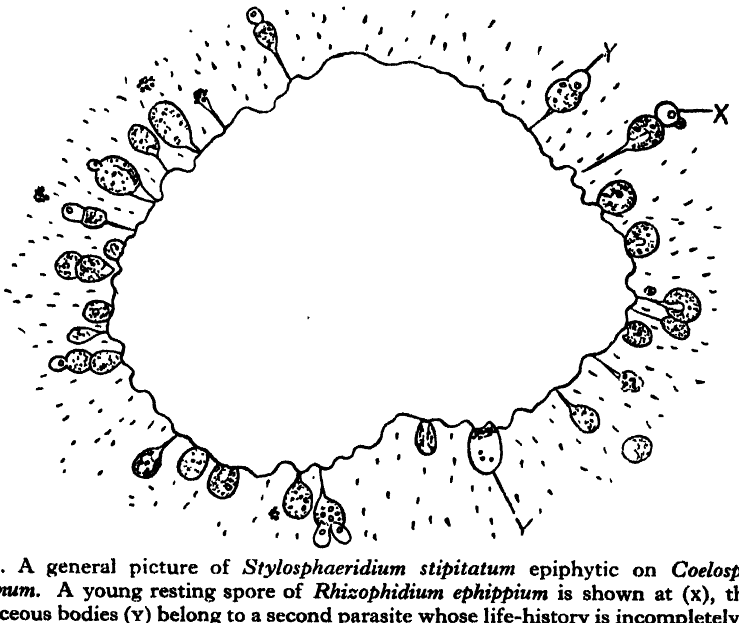

FIG. 5. A general picture of *Stylosphaeridium stipitatum* epiphytic on *Coelosphaerium Naegelianum*. A young resting spore of *Rhizophidium ephippium* is shown at (X), the other chytridiaceous bodies (Y) belong to a second parasite whose life-history is incompletely known. The broken lines indicate bacteria embedded in the mucilage of the *Coelosphaerium*. (× 683.)

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[1] *Stylosphaeridium stipitatum* is an epiphyte on the colonial blue-green alga *Coelosphaerium Naegelianum* Kütz.

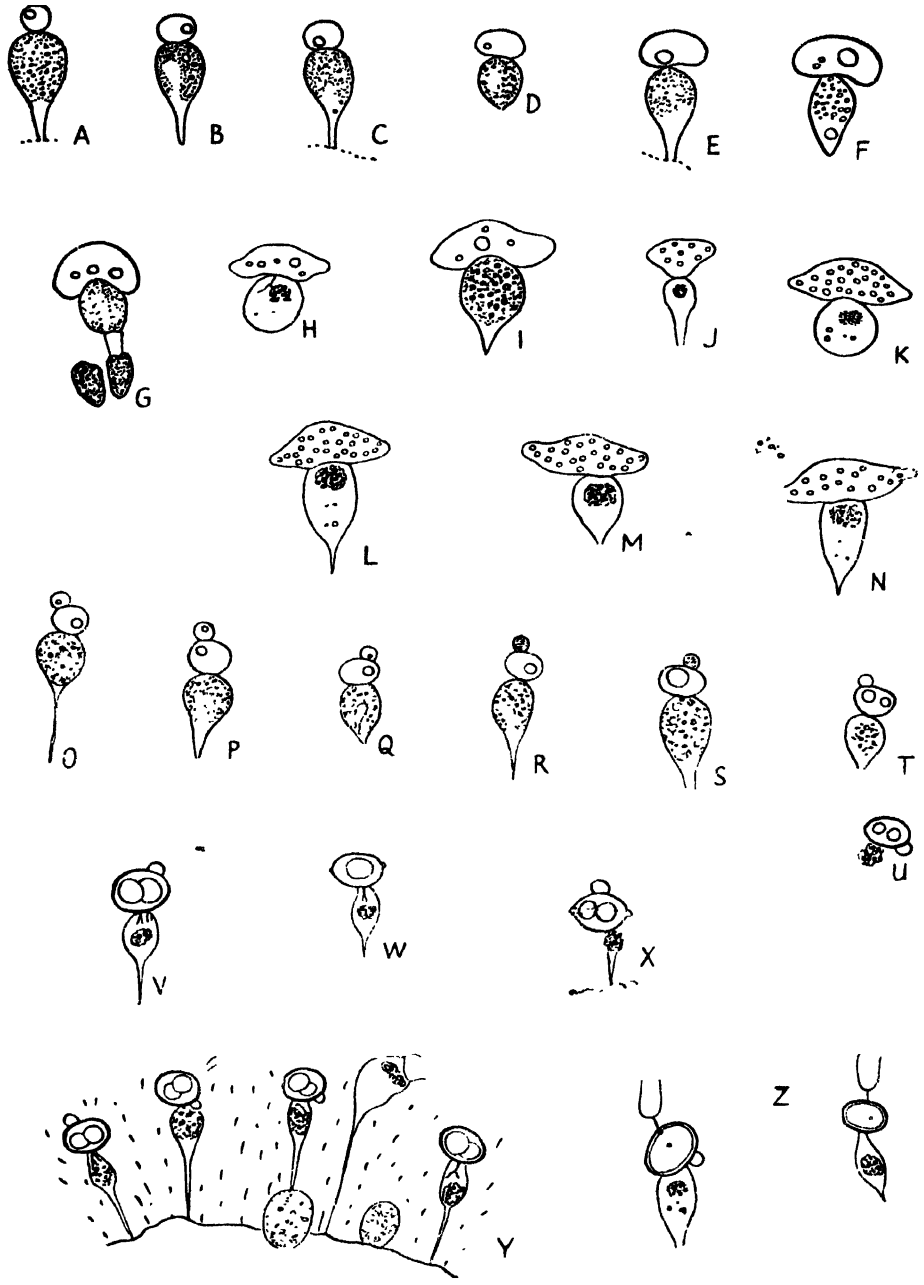

FIG. 6, A–Z. *Rhizophidium ephippium* n. sp. A–I, stages in development from the encysted zoospore to the immature sporangium. J–M, mature sporangia. N, dehisced sporangium with zoospores. O–Q, female cells with recently attached male thalli. R, S, male thalli in which the refractive globule has disappeared but the protoplasmic contents have not completely passed into the female cells. T, empty male cell, wall of female not yet thickened. U–X, mature zygotes; W, viewed from the end, X, shows central girdle. Y, a portion of the mucilage surrounding a *Coelosphaerium* colony (embedded in which are bacteria), showing four *Stylosphaeridium* cells with mature fungal zygotes. Z, two zygotes bearing empty sporangia of a hyperparasite. (A–Z, × 1066.)

during the growth of the sporangium, but at maturity there are from 8 to 30 equal-sized globules each indicating the position of a zoospore. The mature sporangium varies from 8 μ broad by 4 μ high to 15 μ broad by 5 μ high. The majority of the sporangia possess two dehiscence papillae, but occasionally only one is present. On dehiscence the papillae dissolve and the zoospores squeeze their way singly through the openings (Fig. 6, N), passing through the mucilage sheath of the *Coelosphaerium* colony to reach the water. The zoospore is spherical (2 μ) with a small refractive globule and single posterior flagellum. The sporangium wall is very delicate and apparently soon disappears after dehiscence, for only a few empty ones have been observed.

The host chloroplast is always reduced to a mass of reddish granules which are often located immediately beneath the sporangium (Fig. 6, J–N), leaving the remainder of the cell empty. No rhizoids have been seen in this empty region; therefore it is reasonable to suppose that they are not extensive, and in most instances must be buried in the host residue. Only once have I observed any structure resembling a rhizoid connected with a sporangium (Fig. 6, H).

Resting spores are formed by a sexual process identical with that already described for *Rhizophidium goniosporum* Scherffel (1925), *R. fallax* Scherffel, and *R. planktonicum* Canter (1948). The earliest stages in this process are shown in Fig. 6, O–S. The male (essentially an encysted zoospore) attaches itself to a spherical female cell (resembling a slightly enlarged zoospore). The oil globule of the male diminishes in size and finally disappears, so that its protoplasm appears to be evenly granular. This protoplasm must eventually pass into the female, for the male cell adherent to each mature zygote is always devoid of contents (Fig. 6, T, U). The female thallus enlarges after a male has made contact with it, and at maturity it is broadly oval to subspherical in shape, 5 μ broad by 3·7 μ high to 7 μ broad by 5 μ high (Fig. 6, V, Y). The contents consist of two large oil globules, although only one is apparent when the spore is viewed end on (Fig. 6, W). The wall is thickened and often bears a central projecting girdle of unknown composition (Fig. 6, X). The degradation of the host content is similar to that produced by the sporangium. In a number of specimens rhizoids have been observed which take the form of a few short threads (Fig. 6, V, W). Germination of the resting spore has not been seen. Two zygotes were seen bearing empty sporangia of a hyperparasite (Fig. 6, Z).

Although the method of formation of the resting spore is similar to that found in *Rhizophidium goniosporum*, *R. fallax*, and *R. planktonicum*, nevertheless the fungus here described cannot be identified with any of these species. In its sporangial characters it most closely resembles *R. transversum* (Braun) Rabenhorst. A few of Dangeard's (1900–1) drawings of this fungus are reproduced in Fig. 7, A–F. The most obvious difference is found in the asexual nature of the resting spore. Until more is known regarding this structure in *R. transversum* it is suggested that a new species should be erected, *R. ephippium*, taking its name from the saddle shape of the sporangium.

Rhizophidium ephippium sp. nov.

Thalli monocentrici, eucarpici, sporangiis ephippiiformis (8–15μ latis × 4–5μ altis), rhizoideis perpaucis muniti. *Sporangia* poris duobus lateralibus oppositis dehiscentes. *Zoosporae* globosae, 2μ diam., uniguttulatae, postice uniflagellatae. *Sporae* perdurantes ovatae vel subglobosae, 5–7×3·7–5μ, membrana levi hyalina, ex conjunctione cellulae masculae parvae cum cellula femina majore generatae (cellula mascula modo appendiculare persistenti), intus globulis duobus magnis refractis munitae.

Hab.: In cellulis vivis *Stylosphaeridii stipitati* Windermere, Esthwaite Water, et Blelham Tarn, Angliae.

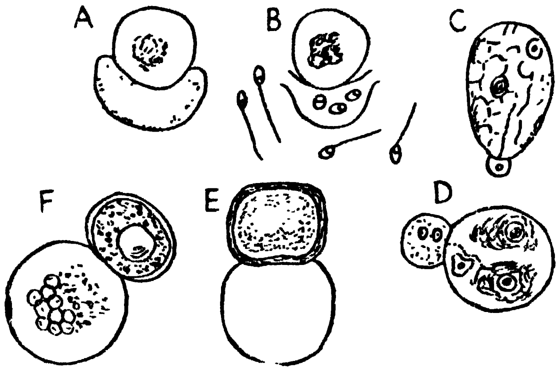

Fig. 7, a–f. *Rhizophidium transversum* (Braun) Rabenhorst. a–f, development and structure of *Chytridium transversum* A. Braun (after Dangeard 1900).

Rhizophidium ephippium n. sp.

Thallus eucarpic, epibiotic, sporangium saddle shaped, 8μ broad by 4μ high to 15μ broad by 5μ high, containing 8 to 30 zoospores. Zoospores spherical, 2μ in diameter, uniguttulate, posteriorly uniflagellate, emerging singly after dissolution of two oppositely directed lateral papillae. Intramatrical rhizoidal system composed of one or a few short threads. Resting spores oval to subspherical, 5μ broad by 3·7μ high to 7μ broad by 5μ high, arising from fusion of the content of a small male with a larger female thallus, the former remaining as an appendage to the mature resting spore. Wall smooth, colourless, beset with a narrow central projecting band of wall material, content consisting of two large refractive globules, germination unknown.

Parasitic on *Stylosphaeridium stipitatum* Geitler from Windermere North and South Basins and Esthwaite Water, in the English Lake District.

III. *CHYTRIDIUM VERSATILE* SCHERFFEL

This fungus occurs together with *Rhizophidium Fragilariae* on *Fragilaria crotonensis*, but never in such large numbers as the latter. Neither *Asterionella formosa* nor *Melosira italica* has ever been observed to be parasitized by this chytrid. The obpiriform sporangium (11·9μ high by 7·4μ at its greatest diameter to 9·5 μ high by 5·7 μ diameter) tapers to a cup-shaped base resting on a short extramatrical stalk 1–2 μ long. The intramatrical rhizoidal system was not observed. At maturity the sporangium contains 20–30 oil globules each indicating the position of a zoospore. Dehiscence of the sporangium is undoubtedly operculate; the convex operculum often remaining close to the empty sporangium (Fig. 8, E, F). The sporangium does not collapse after dehiscence. No resting spores have been found.

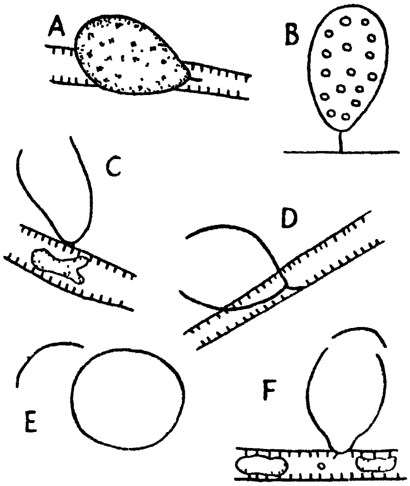

FIG. 8, A–F. *Chytridium versatile* Scherffel. A, immature sporangium. B, mature sporangium. C–F, empty sporangia, operculum present in E and F. (E) viewed from above. (A–F, × 1400.)

TABLE III

Occurrence in the Lake District

Lake.	Host.	1946.	1947.	1948.
Windermere: South Basin	*Fragilaria crotonensis* (A.M. Edw.) Kitton	Sept. 10–Oct. 23	Nov. 11–Dec. 22	Jan. 6 at intervals,–Oct. 26
	Tabellaria fenestrata (Lyngb) Kütz. var. *Asterionelloides.* Grün.	Oct. 8	Nov. 11	June 30–Aug. 3
Windermere: North Basin	*Fragilaria crotonensis*	Oct. 20, Nov. 20	—	July 26–Aug. 3
	Tabellaria fenestrata var. *Asterionelloides*	Nov.	—	—
Esthwaite Water	*Fragilaria crotonensis*	Oct. 28	—	—

IV. *RHIZOPHIDIUM FRAGILARIAE* N. SP.

TABLE IV

Occurrence of R. Fragilariae *on* Fragilaria crotonensis (*A. M. Edw.*) *Kitton*

Lake.	1946.	1947.	1948.
Windermere: South Basin	Sept. 2–Oct. 30, Dec. 12	Feb. 5–June 18, July 4, Nov. 11– Dec. 22	Jan. 6–Nov. 10
Windermere: North Basin	—	Oct. 20–Dec. 1	Apr. 20–Nov. 22
Esthwaite Water	Apr. 18, Aug. 27–Nov. 14	—	Apr. 2–Nov. 16

This parasite (Fig. 9, A–G) appears to be limited to *Fragilaria crotonensis*, as it has never been observed on any other diatom of the plankton. The sessile sporangia are spherical to subspherical and vary greatly in size; small ones, 3μ in diameter, produce about three zoospores, while the largest, 10μ in diameter, may contain as many as twenty zoospores. The majority range from $5{\cdot}2$ to $7{\cdot}7\mu$ in diameter. The intramatrical rhizoidal system, where visible, consists of a short unbranched or once-branched thread. At maturity the sporangium wall deliquesces, forming one, two, or three pores through which the zoospores emerge (Fig. 9, G). At first they are entangled by their flagella, but after a few minutes they swim away individually. The zoospores are spherical, 2–$2{\cdot}4\mu$ in diameter, with a conspicuous anterior oil globule and posterior flagellum. Although many thousands of specimens have been observed no resting spore has yet been found.

In spite of the fact that no distinctive characteristic can be cited for this chytrid it nevertheless seems so common and so definite in its host relations to warrant the erection of a new species. *Rhizophidium Fragilariae* is suggested.

Rhizophidium Fragilariae sp. nov.

Thallus e sporangio externo et parte rhizoidea interna brevi haud vel singulariter ramosa compositus. *Sporangium* globosum vel subglobosum, 3–10μ diam., 3–20 zoosporas includens; tunica in maturitate deliquescenti, et apertiones singulas ad tres, per quas zoosporae abeunt, efformante. *Zoosporae* globosae, 2–$2{\cdot}4\mu$, globulo conspicuo anteriore et flagello singulo simplice posteriore praeditae.

Sporae perdurantes nondum visae.

Hab.: In *Fragilaria crotonensi* (A.M. Edw.) Kitton viventi parasitica in lacubus Esthwaite Water et Windermere, prope Wray Castle, Ambleside, Angliae.

Rhizophidium Fragilariae n. sp.

Sporangia epibiotic, spherical to subspherical, 3–10μ in diameter, containing 3 to 20 zoospores. Sporangium wall deliquesces at maturity, forming 1 to 3 openings through which the zoospores emerge. Zoospore spherical, 2–$2{\cdot}4\mu$, with a conspicuous anterior globule and single posterior flagellum. Endobiotic rhizoid a short unbranched or once-branched thread. Resting spore not observed.

Parasitic on *Fragilaria crotonensis* (A. M. Edw.) Kitton in Esthwaite Water and Windermere, near Wray Castle, Ambleside, England.

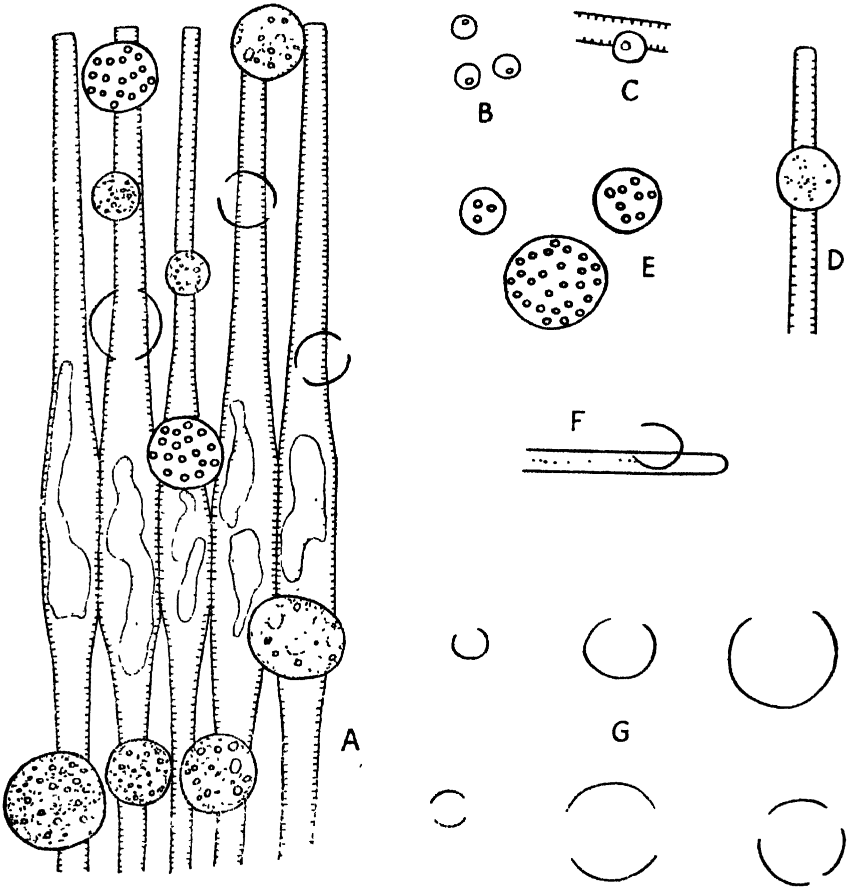

FIG. 9, A–G. *Rhizophidium Fragilariae* n. sp. A, portion of a *Fragilaria* filament bearing young, mature, and empty sporangia. B, zoospores. C, recently encysted zoospore. D, immature sporangium. E, mature sporangia. F, empty sporangium and its rhizoid. G, empty sporangia showing variation in size and number of the dehiscence pores. (A–E, G, × 1400; F, × 1066.)

V. *RHIZOPHIDIUM SPHAEROCYSTIDIS* N. SP.

TABLE V

Occurrence on Sphaerocystis Schroeteri *Chod.*

Lake.	1947.
Windermere: North Basin .	July 8–25
Windermere: South Basin .	July 1–15
Blelham Tarn . . .	July 11–Aug. 19
Esthwaite Water . .	July 4–10

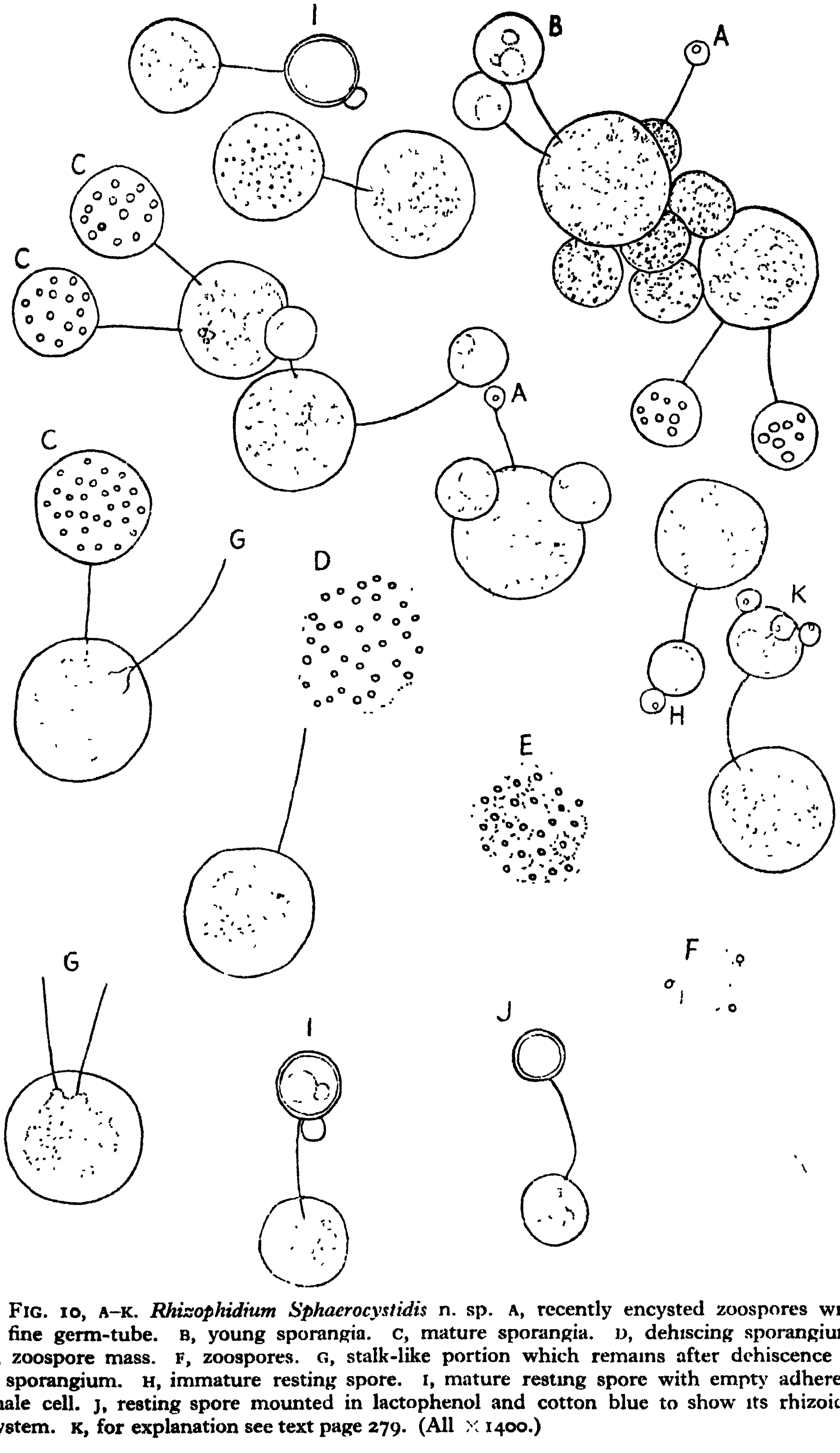

FIG. 10, A–K. *Rhizophidium Sphaerocystidis* n. sp. A, recently encysted zoospores with a fine germ-tube. B, young sporangia. C, mature sporangia. D, dehiscing sporangium. E, zoospore mass. F, zoospores. G, stalk-like portion which remains after dehiscence of a sporangium. H, immature resting spore. I, mature resting spore with empty adherent male cell. J, resting spore mounted in lactophenol and cotton blue to show its rhizoidal system. K, for explanation see text page 279. (All × 1400.)

The encysted zoospore on the mucilage sheath surrounding its host develops a straight rhizoid which penetrates the mucilage, enters the algal cell, and forms inside a meagre branched rhizoidal system, often difficult or impossible to distinguish owing to the dense content of the host. The encysted zoospore enlarges to form a thin-walled spherical sporangium and the germ-tube remains very narrow like a stalk. The mature sporangia (Fig. 10, c) vary from 5 to 11 μ in diameter and contain from 8 to 50 zoospores. On dehiscence the sporangium wall gradually disappears, leaving a protoplasmic mass which becomes irregular (Fig. 10, D) and ultimately cleaves into the zoospores (Fig. 10, E, F). The rhizoid alone indicates the position of a former sporangium (Fig. 10, G). The zoospore is spherical (3 μ), uniguttulate with a long posterior flagellum. The resting spore is sexually formed and develops in a manner already described for *Rhizophidium goniosporum* Scherffel (1925) and *R. planktonicum* Canter (1948). The male gamete attaches itself directly to the wall of the larger female thallus already established on the host cell (Fig. 10, H). The mature zygote is spherical (5–7 μ), with a thick, smooth wall adherent to which is the empty male thallus (2·7 μ) (Fig. 10, I). The content consists of one or two large refractive globules. The presence of the chytridiaceous hyperparasite *Septosperma anomala* (Couch) Whiffen (1942), attacking all stages in

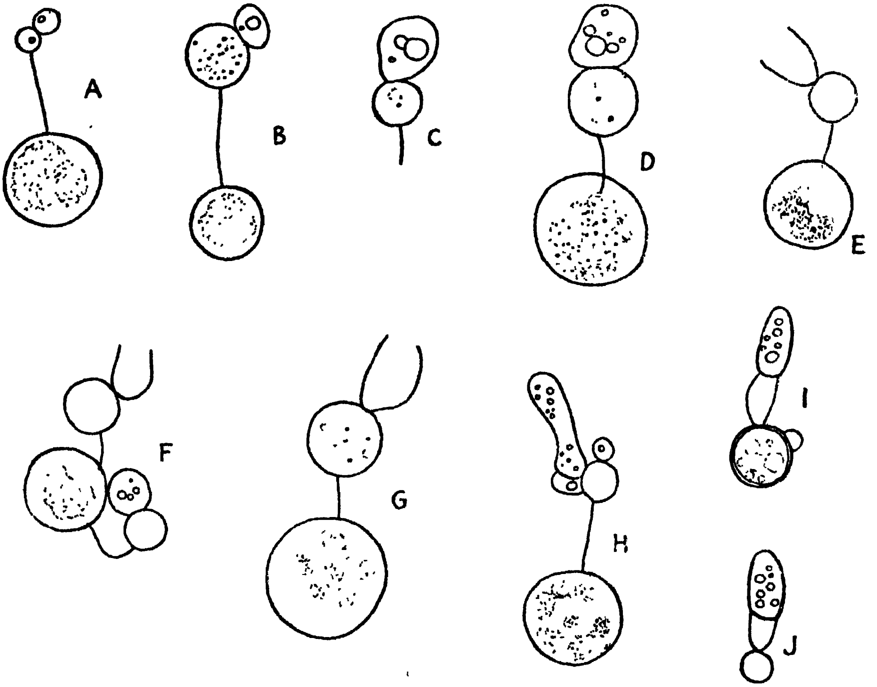

FIG. 11, A–J. *Septosperma anomalum* (Couch) Whiffen. A–D, stages in development of the sporangium. E–G, empty sporangia. H, young resting spore. I, J, mature resting spores. (A–C, F–J, × 1066; D, E, × 1400.)

the life-history of *Rhizophidium Sphaerocystidis*, adds difficulties to the interpretation of the sexual process. Fig. 11, A, can be interpreted in two ways. Either it is the earliest stage in sexual fusion, in which case this fungus would resemble *Phlyctidium Eudorinae* Gimensi (see Sparrow, 1943, p. 153) and *Loborhiza Metzneri* Hanson (1944) where the resting spore is formed after fusion of two isogamous gametes. Secondly, it may be an extreme instance in which a zoospore of *Septosperma* has settled on a zoospore of its host. The latter seems more likely as only one such specimen was observed. Again, it is possible that in Fig. 10, K, the adherent zoospore-like bodies are a mixture of hyperparasite zoospores and male gametes of *Rhizophidium Sphaerocystidis*.

R. Sphaerocystidis differs from the majority of the Chytridiales in the manner of dehiscence of the sporangium. So far three chytrids have been described in which discharge is by dissolution of the whole sporangium wall. They are placed in three monotypic genera, *Nowakowskia* Borzi, *Solutoparies* Whiffen (1942), and *Hapalopera* Fott. In *Nowakowskia* (cited from Sparrow, 1943, p. 288), after dissolution of the sporangium wall, the zoospore mass becomes divided into spore balls, which in their turn break up into smaller spheres. This behaviour is unlike any other chytrid, and I agree with Whiffen (1942) that any species placed in this genus should agree with the type species in this respect.

The difference between *Hapalopera* and *Solutoparies*, however, are not so well marked. While the details of their morphology and their hosts differ, nevertheless they both exhibit a rhizidiaceous type of development. No resting spore is described for *Solutoparies*, and although in the diagnosis of *Hapalopera* Fott (1942) refers to a resting spore as spherical he does not figure or refer to it elsewhere in his paper. However, in a personal communication Dr. Fott kindly sent me a drawing of a resting spore he found in preserved material which I am reproducing with his permission (Fig. 12). No sexual process was observed.

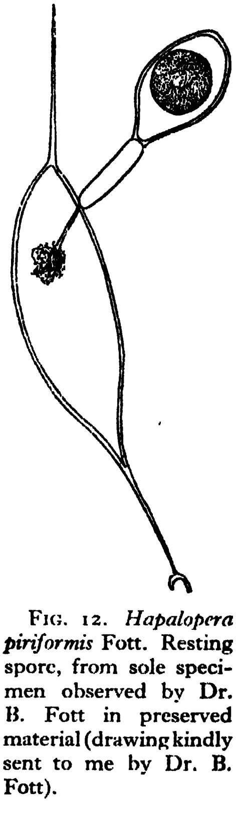

FIG. 12. *Hapalopera piriformis* Fott. Resting spore, from sole specimen observed by Dr. B. Fott in preserved material (drawing kindly sent to me by Dr. B. Fott).

In its method of sexual reproduction my fungus clearly resembles *Rhizophidium goniosporum* Scherffel and *R. planktonicum* Canter. Indeed, these new genera based on the method of dehiscence of the sporangium by deliquescence of the whole sporangium wall may well be unsatisfactory, for in *R. clinopus* Scherffel (1931) the entire upper half of the wall deliquesces, and De Wildeman (1931) sometimes noted total deliquescence of the wall in *R. Schroeteri*. It seems that the fungus here described is new to science, and in spite of its curious method of dehiscence it is suggested that for the time being it should be placed in the genus *Rhizophidium* with the binomial *R. Sphaerocystidis.*

Rhizophidium Sphaerocystidis sp. nov.

Thallus ex partibus tribus compositus; sporangio sessili in vagina mucosa hospitali externe disposito, parte stipitali tereti per vaginam mucosam penetrante, et parte rhizoidea intra cellulam hospitalem ramosa. *Sporangium* globosum (5–11 μ) zoosporas 8–50 includens, tunica in maturitate perfecte deliquescente circumdatum. *Zoosporae* globosae (3 μ), uniguttulatae, ex posteriore uniflagellatae, quandocumque perfecte maturescunt ex sporangio natante. *Sporae perdurantes* globosae (5–7 μ), tunica levi praeditae, globulos oleaceos magnos singulos duosve includentes; per conjugium cellulae masculinae parvae cum cellula femina majore ortae, illa ut appendicula persistente. *Germinatio* nondum visa.

Hab.: In *Sphaerocystide schroeteri*, in lacubus Windermere, Esthwaite Water, et Blelham Tarn, prope Wray Castle, Ambleside, Angliae.

Rhizophidium Sphaerocystidis n. sp.

Thallus consisting of a sporangium sessile on the outside of the mucilage sheath surrounding the host, an unswollen stalk-like region within the mucilage, and a branched rhizoidal system inside the host cell.

Sporangium spherical (5–11 μ) containing 8 to 50 zoospores. At maturity the entire sporangium wall deliquescing and the zoospores when fully delimited swim away. Zoospores spherical (3 μ), uniguttulate, posteriorly uniflagellate.

Resting spores spherical (5–7 μ), wall smooth, containing one or two large oil globules; formed after fusion of a small male cell with a larger female, the former remaining as an appendage to the mature spore. Germination unknown.

Parasitic on *Sphaerocystis Schroeteri* Chod. in Windermere, Esthwaite Water, and Blelham Tarn, near Wray Castle, Ambleside, England.

VI. *ZYGORHIZIDIUM MELOSIRAE* N. SP.

TABLE VI

Occurrence of Z. Melosirae *on* Melosira italica (*Ehrenb.*) *Kütz.*

Lake.	1945.	1946.	1947.	1948.	1949.
Esthwaite Water	Oct. 25	Jan. 5–Apr. 5 Oct. 28, Dec. 30	Jan. 1–Apr. 4 Oct. 16–Dec. 29	Jan. 1–Apr. 12 Oct. 27, Dec. 14	Feb. 15–Apr. 27
Blelham Tarn	Nov. 3–Dec. 24	Oct. 7–Dec. 30	Jan. 6–Apr. 8 Nov. 5–Dec. 30	Jan. 7–Apr. 22 Oct. 28–Dec. 30	Jan. 12–May 9
Windermere: South Basin	—	Feb. 6–Mar. 6	Jan. 8–Apr. 17	Jan. 6–Mar. 16	Mar. 23–Apr. 20
Windermere: North Basin	—	—	—	Jan. 5, Feb. 9, Dec. 13	Jan. 24–May 9
Hawes Water	—	—	—	—	Feb. 3–Apr. 7
Ullswater, West	—	—	—	—	Mar. 3–Apr. 13
Ullswater, East	—	—	—	—	Feb. 3–Apr. 13
Loughrigg Tarn	—	—	—	—	Mar. 21

Both resting and dividing cells of *Melosira* are attacked and the contents reduced to a few small chocolate-brown spheres. The extramatrical sessile or stalked sporangia are ovate and vary from 7 to 14μ high by 5 to 10μ in diameter. The first rhizoid of the encysted zoospore either penetrates the diatom cell immediately or grows for a short distance outside, either free from or running along the surface of the host wall. In examples of the latter the sporangium is often located on a healthy cell, whereas a neighbouring one into which the rhizoids have penetrated is dead (Fig. 13, N).

The Hawes Water form of *Melosira italica* is surrounded by a wide mucilage sheath upon the outside of which the zoospore encysts (Fig. 13, B). A short tube penetrates the mucilage and remains as a stalk to the sporangium.

The intramatrical rhizoidal system is difficult to observe and sometimes cannot be seen, but where visible it consists of a short unbranched or branched thread which does not appear to taper (Fig. 13, N). At maturity the sporangium contains from 6 to 30 relatively large oil globules, each marking the position of a zoospore (Fig. 13, H, I). On dehiscence the unthickened apex of the sporangium separates as a lid. The first few zoospores emerge together and remain a few seconds at the orifice before swimming away; the rest are liberated singly. The zoospore is spherical, 3·3–3·8μ in diameter, with a large oil globule (0·9μ) and a single posterior flagellum. The empty sporangium does not collapse on dehiscence (Fig. 13, J L).

The extramatrical resting spore, 9·5–10μ high by 6·1–7·5μ in diameter, is similar in shape to the sporangium but has a thick, smooth, highly refractive wall and at maturity contains a few large oil globules (Fig. 13, Q, R). It is formed after fusion of the contents of a small male cell (essentially an encysted zoospore) with those of a larger female thallus. The spherical male cell, 3 3·3μ in diameter, is connected to the lateral wall of the female by a conjugation tube, 0·7–7·5μ long by 1·0μ broad (Fig. 13, O -R). The rhizoidal system of the female thallus is like that of the sporangium, but no rhizoids have been seen attached to the male. Where the male is situated on a different host cell from the female, the host content of this cell remains quite healthy. The earliest observed stage in the union between the two thalli is shown in Fig. 13, O. The method of germination of the resting spore is unknown.

This chytrid, by the possession of an operculum and an epibiotic, sexually formed resting spore clearly belongs to the genus *Zygorhizidium*. It differs, however, from the type species *Z. Willei* Löwenthal (1905) in the shape of the sporangium and resting spore and in the absence of a subsporangial swelling, although in the type species the latter is not a constant feature. Scherffel (1925) noted that the non-sexual thalli of the form on *Mougeotia* differed from those on *Cylindrocystis* in the possession of a group of short, rod-like rhizoids, instead of an endobiotic knob. Only one other species has been described, namely *Z. verrucosum* Geitler (1942), in which the wall of the resting spore is covered with wart-like processes. The species here described clearly does not belong to either of the already known ones, and the binomial *Z. melosirae* is suggested for it.

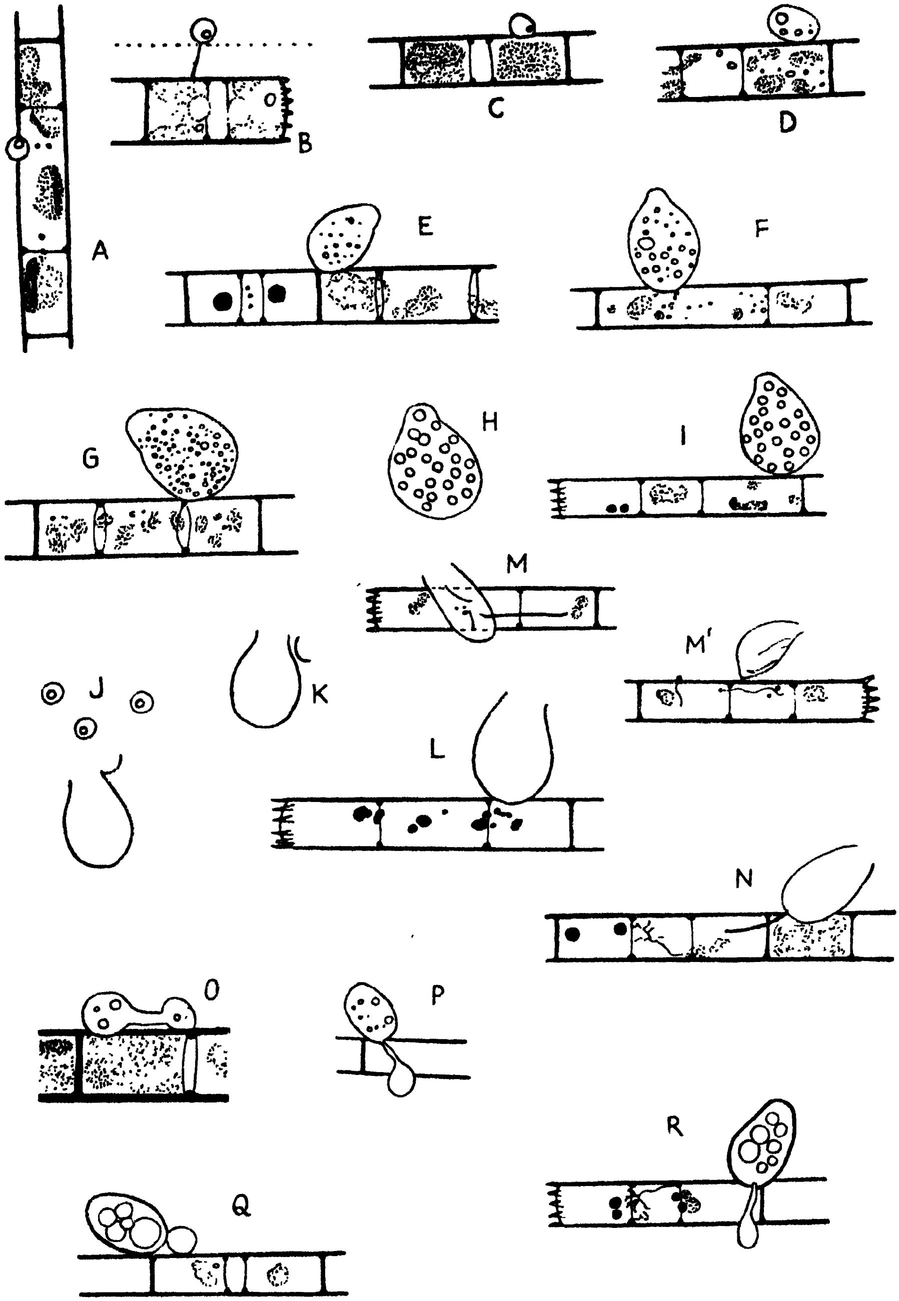

FIG. 13, A–R. *Zygorhizidium Melosirae* n. sp. A, encysted zoospore. B, the same as A but *Melosira* filament is surrounded by a mucilage sheath, which is indicated by a dotted line. C–G, stages in growth of the sporangium. H, I, mature sporangia. J, K, empty sporangia with opercula; zoospores. L–N, empty sporangia. M, M^1, the same sporangia in different views; in M the portion of the rhizoid outside the diatom cell is visible, in M^1 the endobiotic portion. O, young male and female thallus united by a conjugation tube. P, immature resting spore. Q, R, mature resting spores. (B, × 1066; O, × 1400; Rest, × 800.)

Zygorhizidium Melosirae sp. nov.

Thallus epibioticus, sessilis vel pedicellatus. *Sporangium* ovatum, 7–14μ altum, 5–10μ latum, zoosporas 8–30 continente, operculo dehiscente. *Zoosporae* sphaericae, 2·8–3·3μ diam., guttula oleosa magna (0·9μ), postice uniflagellatae. *Rhizoideum* intramatricale, simplex vel sparsim ramosum, aequale. *Sporae perdurantes* ovatae 9·5–10μ altae, 6–7·5 latae, laeves, intus paucis globulis praeditae, per conjugium cellularum sexualium ortae, cellula mascula parva cum cellula feminea majore per tubulum 0·7–1·5μ longum conjugante. *Germinatio* nondum visa.

Hab: In *Melosiram italicam* parasiticus, Esthwaite Water, Blelham Tarn, North Basin et South Basin, Windermere, Ullswater, Hawes Water et Loughrigg Tarn, Anglia.

Zygorhizidium Melosirae n. sp.

Thallus epibiotic, stalked or sessile, sporangium ovate 7–14μ high by 5–10μ in diameter with 6 to 30 zoospores, dehiscing by a lid. Zoospores spherical, 2·8–3·3μ in diameter, with a large oil globule (0·9μ), and posterior flagellum. Intramatrical rhizoid unbranched or sparingly branched, not tapering. Resting spores ovate, 9·5–10μ high by 6–7·5μ broad, wall smooth, the content with a few large globules; arising from fusion of the contents of a small male with a larger female cell by a conjugation tube, 0·7–7·5μ long. Germination unknown.

Parasitic on *Melosira italica* in Esthwaite Water, Blelham Tarn, Windermere North and South Basins, Ullswater, Hawes Water and Loughrigg Tarn, the English Lake District.

VII. ZYGORHIZIDIUM PARVUM N. SP.

TABLE VII

Occurrence of Z. parvum *in the English Lake District*

Lake.	Host.	1947.	1948.	1949.
Blelham Tarn	*Sphaerocystis Schroeteri* Chod.	June 19–Sept. 10	May 14–July 22	—
	Kirchneriella obesa (W. West) Schmidle	July 11	—	—
Esthwaite Water	*Sphaerocystis Schroeteri*	June 26–Aug. 28, Oct. 16	Apr. 20–July 28	—
Windermere: South Basin	*Sphaerocystis Schroeteri*	July 1–15	June 16–July 14	Apr. 6–May 10
Windermere: North Basin	*Sphaerocystis Schroeteri*	July 8–25	June 1–July 12	Apr. 19–May 9

Sphaerocystis Schroeteri and *Kirchneriella obesa* are both surrounded by a wide mucilage sheath. The sporangium develops from the encysted zoospore and a part or the whole of the germ-tube (Fig. 14, A, B). When mature it varies

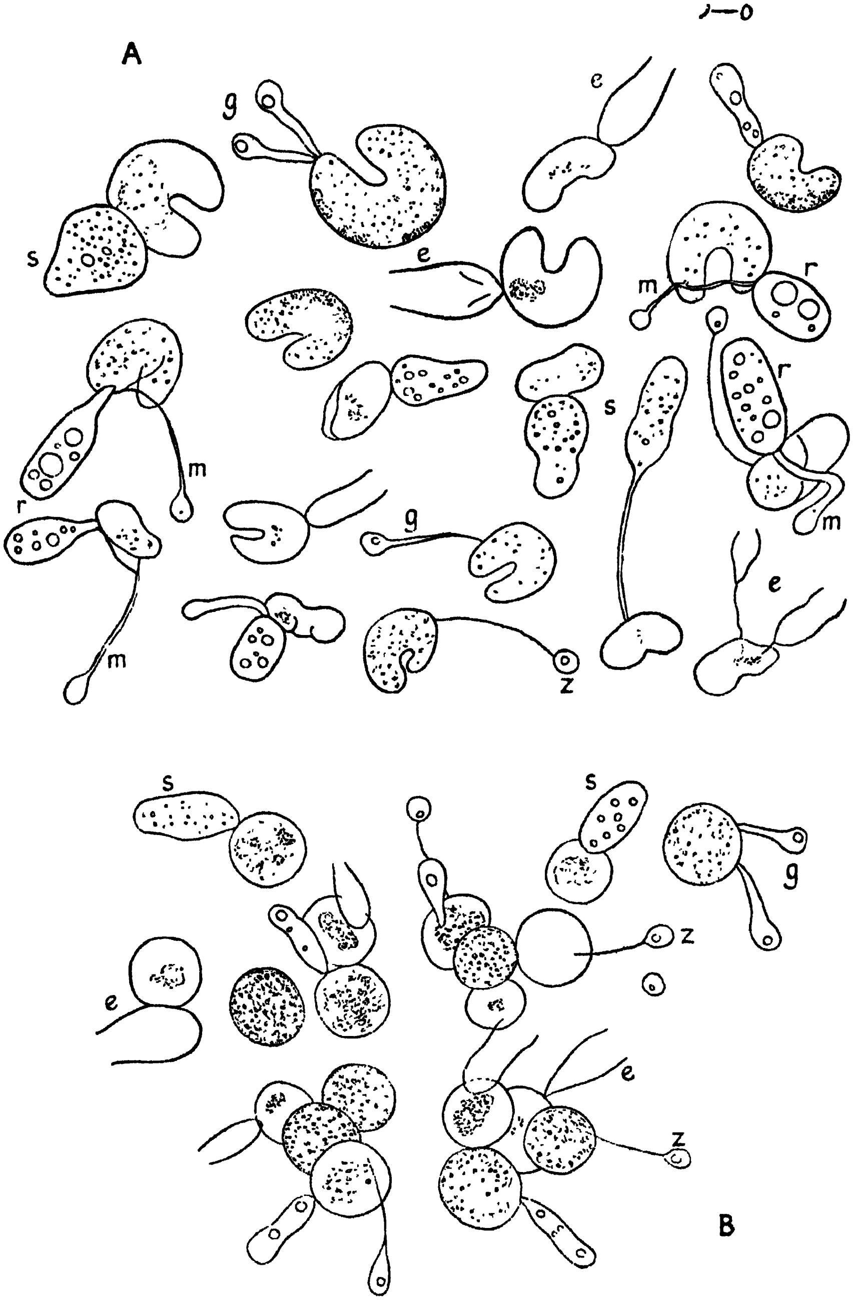

FIG. 14, A, B. *Zygorhizidium parvum* n. sp. A, *Kirchneriella*, B, *Sphaerocystis* cells bearing the parasite in various stages of development. *e*, empty sporangia; *g*, broadening of germ-tube; *m*, male thallus; *o*, operculum; *r*, resting spore; *s*, sporangia; *z*, encysted zoospore with germ-tube. (× 1400.)

in shape from piriform, measuring 4·7μ high by 2·3μ broad to 13μ high by 8μ broad, to cylindrical, 9·5μ high by 1·9μ broad. The sporangium may be sessile on the host cell or stalked. If there is a stalk it is simply part of the original germ-tube which has not swollen. Both mother and daughter algal cells are parasitized, the mother cells bearing the larger sporangia. There are from 4 to 40 zoospores in a sporangium, and when it is narrow and elongate the zoospores are arranged in a linear series. The zoospores are fully formed within the sporangium, and several emerge together when the lid becomes detached; the rest follow in rapid succession. The zoospores (1·9μ in diameter) are spherical, with a relatively large anterio-lateral globule and accompanied by a much smaller one.

The rhizoidal system appears to be of limited extent and where visible consists of a small unbranched thread.

Resting spores formed by a sexual process are produced in quantity. Their method of formation is similar to that described for *Zygorhizidium* spp. (see Löwenthal, 1905; Geitler, 1942) and *Rhizophidium columnaris* Canter (1947). Contact is established between a minute male thallus (essentially an encysted zoospore and its germ-tube) with a slightly larger female thallus by means of a conjugation tube which arises as a lateral branch from the male, passing through mucilage until it reaches a female (Fig. 15, H–L). Although it is presumed that the germ-tube of the male enters the host cell to which it is attached, no internal rhizoidal system has been observed and such host cells show little sign of disease. The content of the male passes into the female, which then enlarges into the resting spore. The fusing gametes are very slightly different in size (Fig. 15, H, I). At first the resting spore contains a few large oil globules, but when mature there are more numerous smaller globules. The resting spore may be spherical (6–8μ) or elongate oval (5·3μ high by 3·5μ broad to 15μ high by 8μ broad); it is always surrounded by a thick, colourless, smooth wall (Fig. 15, L).

As has been recorded for *Zygorhizoidium Willei* Löwenthal (1905) and *Rhizophidium columnaris* Canter (1947), male thalli which have found no female with which to fuse may then grow into sporangia (Fig. 15, M).

One other parasite has been recorded on *Sphaerocystis Schroeteri* from the plankton of the Walensee by Huber-Pestalozzi (1946, p. 99, fig. 6); see Fig. 16. Huber-Pestalozzi, however, neither identified it with a known species nor described it under a new binomial. It may be identical with the British form since the sporangia are of similar shape and develop in the same manner. However, no resting spores were observed, nor is it known whether the sporangium is operculate.

Once again difficulties are presented in relation to the generic position of this chytrid. Although the method of formation of the resting spore is similar to the operculate *Zygorhizidium*, nevertheless it differs in the fact that both the sporangium and resting spore develop from the encysted zoospore and its germ-tube. The fungus under consideration is parallel with *Dangeardia mammillata* (Canter, 1946) among the inoperculate chytrids.

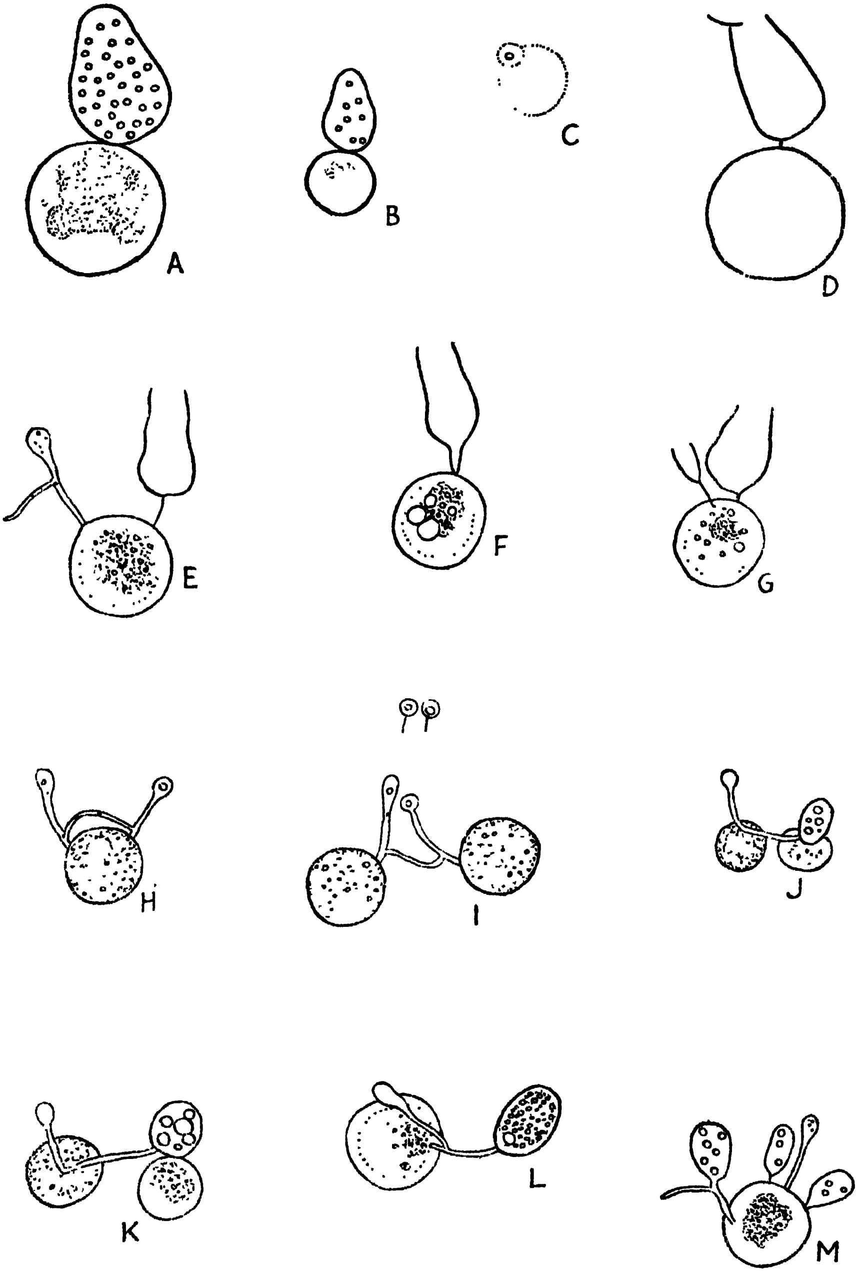

FIG. 15, A–M. *Zygorhizidium parvum* n. sp. A, B, mature sporangia. C, zoospore. D–G, empty sporangia. E, empty sporangium and male thallus which shows signs of developing into a sporangium. H, I, conjugating thalli; a slight anisogamy of the gametes is noticeable. J, K, immature resting spores. L, mature resting spore. M, host cell bearing small sporangia, and a male thallus which has developed into a zoosporangium. (A–D × 1400; E–M × 1130.)

Although it may be that a new genus of the operculate series should be erected for this fungus, in our present incomplete knowledge of these forms and characters on which to base generic distinction it seems best to include it in the genus *Zygorhizidium* as a new species *Z. parvum.*

Zygorhizidium parvum sp. nov.

Thalli monocentrici, eucarpici, sporangiis et rhizoideis brevibus tenuibus simplicibus muniti. *Sporangia* piriformia, cylindrica vel ovata, 5–13 × 2·5–8μ, per operculam dehiscentes, zoosporis 4–40, postice uniflagellatis, 2μ diam. *Sporae perdurantes* globosae, 6–8μ diam., vel ovatae 5·3–15 × 3·5–8μ, membrana levi, multiguttulatae, sexualiter formatae (ut in aliis speciebus generis) sed thalli masculi tubo conjugationis laterali e tubo germinationis emergenti.

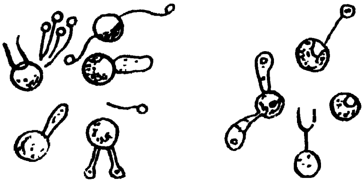

FIG. 16. Parasite on *Sphaerocystis Schroeteri* Chod. from the Walensee (after Huber-Pestalozzi 1946 Abb. 6).

Hab.: In cellulis vivis *Sphaerocystidis Schroeterii*, Blelham Tarn, Esthwaite Water et Windermere, et in cellulis vivis *Kirchneriellae obesae*, Blelham Tarn, prope Wray Castle, Angliae.

Zygorhizidium parvum n. sp.

Thallus monocentric, eucarpic, consisting of a sporangium developed from the body of an encysted zoospore and the whole or a part of the original germ-tube. Sporangia pear-shaped, cylindrical, or oval, varying in size from 5 to 13μ high by 2·5 to 8μ broad. Zoospores from 4 to 40 fully formed within the sporangium, emerging after the detachment of a lid. Zoospores, 2μ in diameter, posteriorly uniflagellate, with an anterior lateral globule and a minute shining granule. Rhizoid a short, unbranched thread. Resting spore sexually formed male thallus (resembling an encysted zoospore and its germ-tube) makes contact with a slightly larger female thallus by means of a conjugation tube which arises as a lateral branch from the germ-tube of the male. Resting spore spherical, 6–8μ in diameter, or elongate-oval, 5–15μ × 3·5–8μ. Wall smooth, hyaline, content composed of small globules. Germination unknown.

Parasitic on *Sphaerocystis Schroeteri* Chod. in Blelham Tarn, Esthwaite Water, and Windermere, and on *Kirchneriella obesa* (W. West) Schmidle, in Blelham Tarn, near Wray Castle, the Lake District, England.

RECENT RECORDS OF PHYTOPLANKTON PARASITES FROM OTHER COUNTRIES

The fungal parasites of planktonic algae have received little attention from students of chytrids, and particulars of their distribution are few. In recent years two workers who are primarily algologists have described parasites from Switzerland and Sweden. Huber-Pestalozzi (1946) figures chytrids on *Asterionella formosa* Hass and *Sphaerocystis Schroeteri* Chod. from the Walensee and on *Fragilaria capucina* from Zürichsee. Earlier this worker (1944) describes *Chytridium oocystidis* n. sp. parasitizing *Oocystis lacustris* Chod. in the Walensee.

The Swedish records are by Skuja (1948, pp. 379–82). *Olpidium entophytum* (A. Braun) Rabenhorst is described in *Gloeocystis bacillus* and *Gloeocystis planktonica* from Erken.

A very similar parasite occurs in *Gloeocystis* in the English Lake District. All the details of its life-history are not known, but it produces biflagellate zoospores. Skuja never observed the zoospores in his form and it is possible, especially from his drawing (1948, Taf. XXXIX, Fig. 7), that this species is also a biflagellate and does not belong in the genus *Olpidium*. *Olpidium endogenum* (Braun) Schroeter is described from Arnsjou in *Cosmarium depressum* var. *achondrum*. Skuja does not refer to the zoospores of this fungus and again it may be biflagellate, in which case it could be referred to a dwarf form of *Myzocytium megastomum* De Wildeman.

Phlyctidium anabaena Rödhe and Skuja is a new species parasitic in young resting spores of *Anabaena* spp. from Säbysjön. *Chytridium microcystidis* Skuja parasitizes *Microcystis* spp. in Erken.

The remaining parasites occur on *Melosira ambigua*. They are *Rhizophidium simplex* (Dang.) Fischer from Erken, *Rhizophidium fusus* (Zopf) Fischer from Örsjön, and *Chytridium versatile* Scherffel from Örsjön, Bredsjön, and Ubby-Långsjön.

It is unlikely that the lakes in the English Lake District are exceptional in the large variety of algal parasites they contain. It is probable that if other lakes with a comparable algal flora were constantly studied, they too would be found to possess a similar parasitic fungal flora.

Encouraging results have been obtained from observations on single plankton samples[1] (preserved in formalin) from twenty-five lakes in Switzerland. In ten of these samples parasites were present.

In the Pfaffikersee one specimen of a polyphagous chytrid was found on *Gemellicystis* and on *Sphaerocystis*, a fungus identical with one figured by Huber-Pestalozzi (Fig. 16) from the Walensee. It is possible that these fungi are *Rhizidium windermerense* (p. 265) and *Zygorhizidium parvum* (p. 283) respectively. Again, in this sample *Eudorina elegans* was heavily parasitized by a fungus resembling *Endocoenobium Eudorinae* Ingold (1940).

Although the value of preserved samples is limited for obtaining a sound knowledge of any one fungus life-history, these nevertheless do indicate that in Switzerland there is a wide field open to the enthusiastic aquatic mycologist.

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[1] Kindly collected for the International Limnological Congress in Zürich, 1948, under the supervision of Professor O. Jaag.

SUMMARY

The phytoplankton of four bodies of water in the English Lake District have been examined from October 1946 to April 1949 for the presence of aquatic fungi. A survey of seventeen other lakes was begun in January 1949. Forty different fungi have been observed, seven of which are described in this paper, namely, *Rhizophidium ephippium* n. sp., *R. Fragilariae* n. sp., *R. Sphaerocystidis* n. sp., *Rhizidium windermerense* n. sp., *Chytridium versatile* Scherffel, *Zygorhizidium Melosirae* n. sp., and *Z. parvum* n. sp.

My thanks are due to Mr. D. Gawen for collecting the samples from Windermere, Esthwaite Water, and Blelham Tarn, and especially to Professor C. T. Ingold for his criticism of the manuscript and for all the encouragement he has given throughout the course of this work. For the Latin diagnoses my thanks are due to Miss E. M. Wakefield of Kew, and to Mrs. F. Balfour-Brown of the British Museum.

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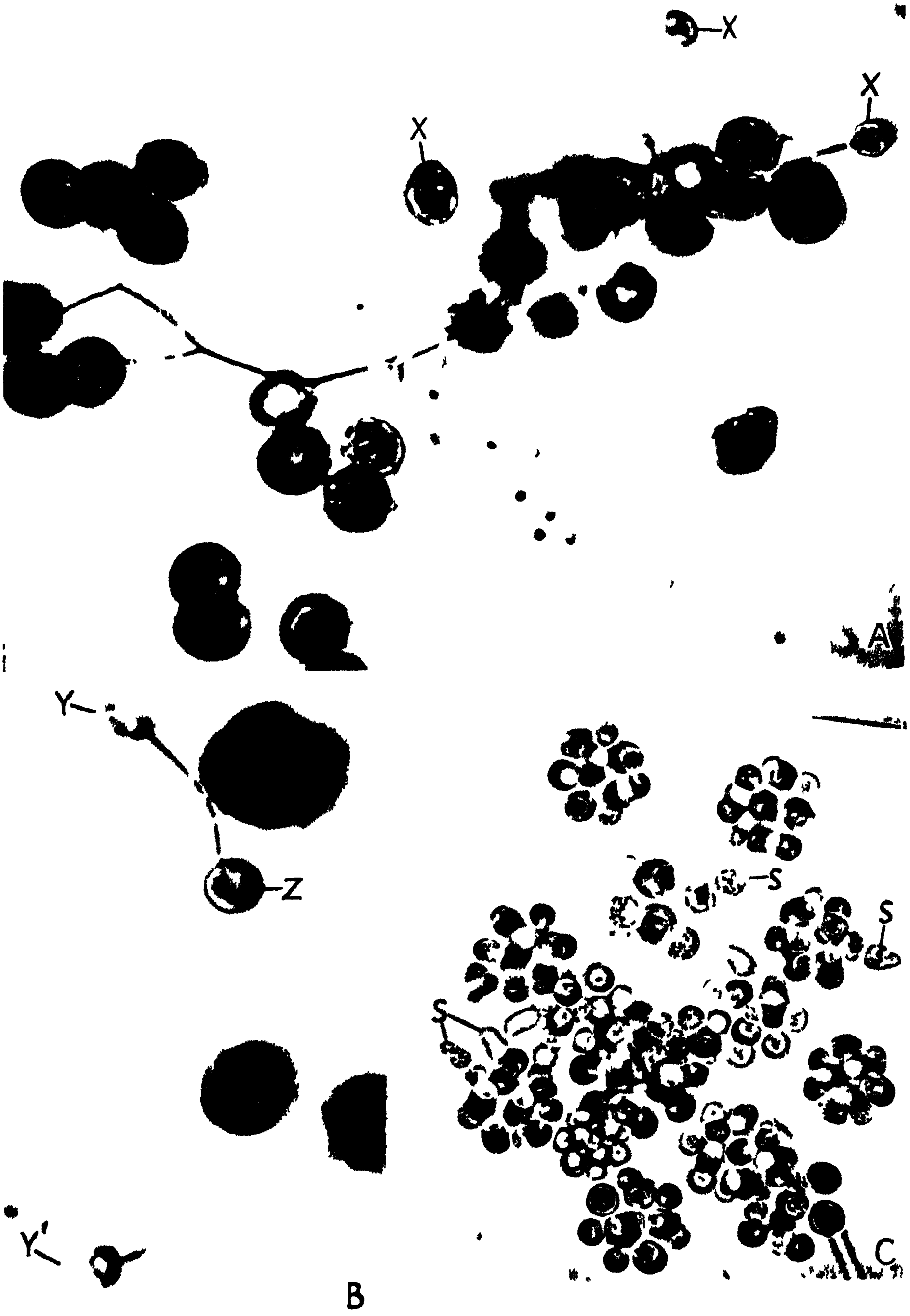

A Part of a colony of *Gemellicystis neglecta* bearing three sporangia (X) of *Rhizidium windermerensis* n. sp. The extensive rhizoidal system is clearly visible ×1764

B *Rhizidium windermerensis* n. sp. Two conjugating thalli (Y, Y′). The spherical body (Z) is the incipient resting spore or zygote produced at the point of fusion of the conjugation tubes ×2200

C Several pear-shaped sporangia (S) of *Zygorhizidium parvum* n. sp. on *Sphaerocystis Schroeteri* ×600

Growth Analysis of Cacao Seedlings

BY

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(West African Cacao Research Institute, Tafo, Gold Coast)

With six Figures in the Text

THE programme of physiological investigations on cacao (*Theobroma cacao*) planned at the West African Cacao Research Institute includes a detailed study of the effects of external factors, particularly light, on seedling growth. With this in view, it was necessary to study the variability of the various changes by which growth can be expressed, to develop methods by which the precision of estimates of these changes could be improved, and to determine the degree of replication which would be required. Hence a series of preliminary observations was conducted under ordinary field conditions, without differential treatments, which would give information as to the normal growth characteristics of cacao and at the same time enable the questions mentioned to be answered.

The present study is an account of these preliminary observations, which extended over a period of 7 months from planting; it appears to be the first such investigation performed on a tree crop. An exhaustive study, on similar lines, of the first 6 weeks of development of the cacao seedling has already been published (Goodall, 1949), and hence the present paper will concern itself mainly with the period between 6 weeks (when the first 'flush' of leaves has almost completed expansion and the cotyledons are nearly ready to fall) and 7 months from planting. It should be explained that the leaves of cacao do not expand in regular succession, but that a group of leaves formed by the same apex expand almost simultaneously, and that an interval of several weeks then elapses before the expansion of the next 'flush'. During the period covered by these observations a total of either five or six 'flushes' expanded on each plant.

MATERIAL AND METHODS

Planting material consisted of seeds taken from two pods of each of four West African Amelonado trees. The testas were removed, and 55 seeds taken at random from the progeny of each parent were weighed. The 220 seeds were planted on August 16, 1946, in individual baskets in a nursery under *Leucaena glauca*. This leguminous shade tree, native to America, is commonly used in nurseries and young plantations of cacao, and the light intensity under it was some 20 per cent. of full daylight.

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[1] Now at Botany School, University of Melbourne, Australia.

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Seeds from each parent were taken at the time of planting for dry-weight determinations. The mean loss in weight on drying was 25·8±0·9 per cent., and did not vary with parentage.

The baskets were inspected daily and the date of emergence from the soil noted in each case, as well as that on which the cotyledons separated and the plumule became visible. Shortly after this daily measurements of the stem height (from the cotyledons) and of the length and maximum breadth of each growing leaf commenced.

Plants were harvested at 6, 12, 18, 24, and 30 weeks from planting. On each of the first four occasions 10 plants of each progency were selected, but for the last harvest only 5 plants of each progeny were available. Apart from a small number of failures in germination, a considerable amount of insect damage occurred after 3 months, and the more severely damaged plants were rejected.

At harvest the plants were brought in their baskets to the laboratory and the stem of each was severed immediately below the cotyledonary node. The roots were carefully washed. The leaves were separated and cleaned with a brush. Each leaf, stem, and root, and the cotyledons while they were present, were weighed separately, dried in a steam-oven for at least 24 hours, and re-weighed. Between weighing and placing in the oven a print or outline was made of each leaf for subsequent area determination by the methods already described (Goodall, 1947, 1949).

In addition to these records an attempt was made to collect any parts lost by the plants during development—the cotyledons, when they abscissed at about 8 weeks; young leaves which frequently also fall by abscission before reaching maturity; and leaves lost through insect damage. It was unfortunately impracticable to estimate, day by day, the loss of leaf material through leaf-eating insects removing a part of the lamina.

The principal purpose of the work being to estimate, as closely as possible, the changes in dry weight and amount of water in the plant during successive 6-week periods, and their distribution through the plant, the methods used for estimating these changes must be described in some detail. Since cacao seedlings, like the mature trees, are highly variable, comparison of the uncorrected weights of successive samples was considered to give insufficient precision for the purpose in hand, and methods were sought for providing more accurate estimates of the changes taking place. The principle adopted in most instances was to use the weights and linear measurements of the plants forming one harvest to establish a relationship between these quantities, and then to use measurements made at the same time on plants harvested subsequently to estimate the weight for each of these plants at the time of the first harvest. The difference between this estimate of the initial weight and the weight actually determined 6 weeks later then provided the estimate of the change in weight during that period. This technique was essentially the same as that used for estimating dry-weight changes in tomatoes (Goodall, 1945), though in that case the periods over which changes were measured were of a few hours only. In the present study, as in that with tomatoes, several possible ways of using the preliminary measurements for estimating initial weights were tested, and those selected as giving the lowest errors of estimation are detailed below:

Dry weight. The dry weight at planting of the seedlings harvested at 6 weeks was estimated from the fresh weight of the seeds, together with their mean percentage dry weight. The mean proportions of radicle and cotyledons in other series of observations (Goodall, 1949) were taken as applicable.

Estimates of dry weight of leaves and stems at 6 weeks and subsequently were based on their linear dimensions on the days preceding harvests (or on the last measurement before that date, if the leaves were not then growing). For *mature leaves* the ratio of dry weight to product of length and breadth on the day preceding harvest was found to be independent of leaf size, and thus the weight of a mature leaf at the beginning of an experimental period was estimated from the product of its length and maximum breadth, together with the mean value of this ratio for plants harvested at that time. For *immature leaves*, which at any harvest were relatively few in number, the relationship between dry weight and the product of length and maximum breadth, though close, could not be fitted satisfactorily by a simple mathematical expression, so freehand curves were drawn connecting these values at each harvest, and the lengths and maximum breadths of immature leaves of the subsequent harvest were used to read from these curves their estimated initial dry weight. The dry weights of *stems* were estimated from a linear regression of log. dry weight on log. length. In the case of the *cotyledons* it was found that the proportion of dry matter lost by them during the 6 weeks following germination was related to the total amount of foliage-leaf material at the end of this period; accordingly the regression of the ratio of cotyledon dry weight at 6 weeks to seed fresh weight at planting on the sum of the products of leaf length and maximum breadth at 6 weeks was used to estimate the dry weight at that time of cotyledons subsequently lost by abscission. A linear regression of *root* dry weight at 6 weeks on seed fresh weight at planting was used to estimate the dry weight at that time of roots of plants harvested at 12 weeks. At 12 weeks and subsequently root dry weight could more accurately be estimated from a regression of log. root dry weight on log. stem length.

The dry weight of the *whole plant* was on each occasion estimated as the sum of the estimates of dry weight of the individual organs.

Leaf area. In view of the difficulty of measuring the areas of large numbers of small leaves while still attached to the plant, and the risk of damage, area measurements were made only on leaves separated from the plants at harvest-time. Areas of leaves of plants not harvested were estimated from a linear regression of area on the product of length and maximum breadth.

Water weight. The weights of water in individual *leaves* at 6 weeks and subsequently were estimated from linear regressions of weight of water on product of length and maximum breadth, those in *stems* from linear regressions of log. weight of water on log. stem length. At 6 and 12 weeks the weight of water in the *roots* was estimated from linear regressions of the values for plants harvested at those times on seed fresh weight at planting; at 18 and 24 weeks a more accurate estimate of the weight of water in the roots was given by a linear regression on the sum of the products of length and maximum breadth for each leaf of the plant. In the *cotyledons* the weight of water at 6 weeks was likewise estimated from a linear regression on the sum of products of leaf length and maximum breadth.

As in the case of dry weight, the weight of water in the *whole plant* was estimated by summing the estimates for the various organs.

Errors of estimation. The errors involved in the methods of estimation described were found by using them to estimate the 'changes' between duplicate samples harvested simultaneously. In the 6-week and 12-week harvests alternate plants were used for the determination of parameters, which were then used for estimating the 'changes' in dry weight and water weight of the organs of the other twenty plants in the same harvest. In Table I are shown the root mean squares of the 'changes' thus determined, together with the mean weights of the organs:

TABLE I

Errors of Estimation of Changes in the Weight of a Plant and its Parts

		6 weeks.		12 weeks.	
		Observed mean (mg.).	Root mean square deviation.	Observed mean (mg.).	Root mean square deviation.
Dry matter:	Leaves . .	215	20	607	72
	Stem . .	50	7	159	43
	Roots .	275	28	515	80
	Cotyledons .	596	56	—	—
	Whole plant .	1,136	54	1,281	153
Water:	Leaves . .	614	71	1,193	117
	Stem . .	235	27	538	97
	Roots . .	1,602	195	2,130	337
	Cotyledons .	1,361	200	—	—
	Whole plant .	3,812	301	3,861	373

It will be seen that in most cases the error of estimation for organs of a single plant is between 10 and 15 per cent. of the mean weight. Thus, for a sample of forty plants harvested, the errors of estimation of the mean changes of weight were of the order of 2 per cent. of the initial weight.

As examples of the increase in precision with which changes are estimated by this procedure, Table II shows the changes in weight of water in the plant and its organs between 6 and 12 weeks from planting, as estimated by the methods described, and also by simple comparison of the means of successive samples without taking the concomitant measurements into account. The considerable reduction in the standard errors for leaves and stem is clear. In this particular set of data there was no improvement in precision in the estimate for the cotyledons, and that for the roots did not reach significance;

nevertheless the change in the plant as a whole was estimated much more precisely when the concomitant measurements were used.

In Table II, as also in subsequent tables, variance between progenies has been eliminated from the error estimates. Except where explicitly mentioned it proved to be non-significant. The figures quoted for error between progeny means do not, of course, include any contribution on account of the errors in the parameters used for the estimations—the same parameters having been used for all progenies; the error estimates appropriate to comparisons between different harvests are consequently somewhat greater—usually by no more than 5 per cent.—and the necessary adjustments have been made in determining the significance levels quoted in the text.

TABLE II

Changes in Weight of Water in Plant between 6 and 12 Weeks Estimated by Two Methods

	Without use of concomitant measurements.		With use of concomitant measurements.	
	Mean estimate of change (mg.).	S.E. of mean.	Mean estimate of change (mg.).	S.E. of mean.
Leaves .	+ 453·4	72·2	+ 581·2	41·8
Stems .	+ 231·9	37·2	+ 266·5	20·5
Roots . .	+ 570·3	56·9	+ 604·7	49·9
Cotyledons .	− 19·8	37·6	− 62·5	41·9
Whole plant (incl. cotyledons) .	+1235·8	133·1	+1389·9	100·2

RESULTS

Dry weight

The main features of the dry-weight changes are presented in Fig. 1. The numerical data used in preparing this diagram are composite—that is, the values shown for the harvest at 6 weeks are the actual means for these plants, and those at planting the estimated initial values for the same plants; the values on subsequent occasions, however, were obtained by adding the estimated increases during each period to the actual weights of the respective organs harvested at 6 weeks. The weights of organs lost by abscission are shown in the narrow intermediate columns; these losses might occur, of course, at any time during the 6 weeks separating successive harvests. The cotyledons, which form the major part of these losses, were, on average, shed 68 days after planting.

Over the first 12 weeks of growth the net increase in plant dry weight is negligible—that is, the gain in weight of the whole plant including cotyledons is little more than the weight of the latter at the time of abscission. Thereafter, however, the gain in dry matter is approximately exponential, the dry weight having nearly doubled after each interval of 6 weeks.

In Table III are given the mean relative growth rates for plants of each parentage over each period. For the period 6–12 weeks the dry weight of the cotyledons at abscission has been included in the final plant dry weight.

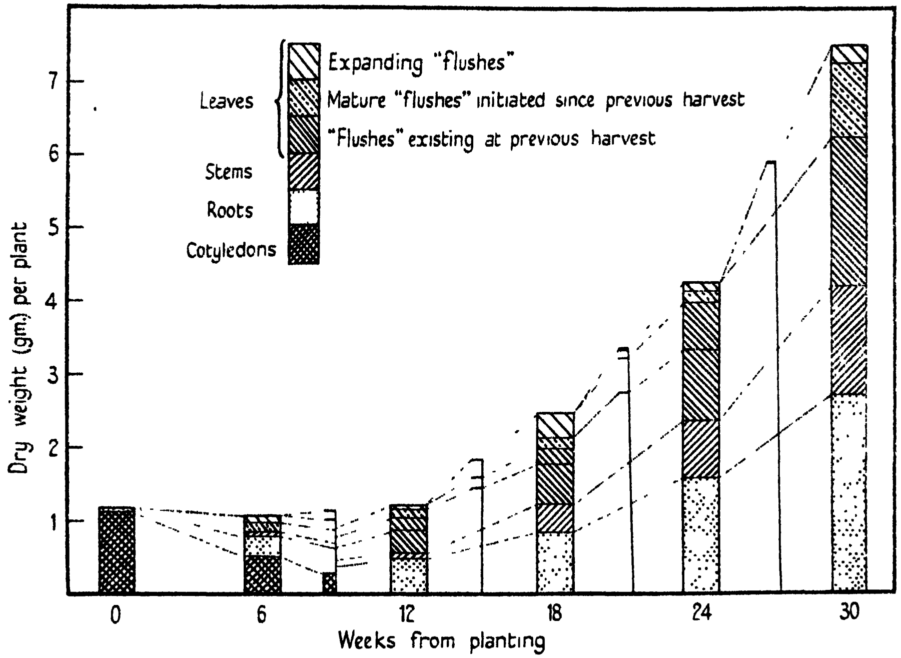

FIG. 1. Increase in dry weight of the cacao seedling and its parts during the first 30 weeks after planting. The narrow intermediate columns represent organs shed during the inter-harvest periods.

TABLE III

Mean Relative Growth Rates (% per day)

Female parent:	E54/122.	E62/129.	E62/31.	E61/93.	Mean.
6–12 weeks . . .	0·71	0·73	0·80	0·64	0·72
12–18 weeks . . .	1·28	1·79	2·07	1·93	1·77
18–24 weeks . . .	1·03	1·28	1·40	1·52	1·31
24–30 weeks . . .	1·04	1·05	1·42	1·85	1·34
Mean, 6–30 weeks . .	1·02	1·21	1·42	1·49	1·29

S.E. of progeny means, 24–30 weeks: 0·14
„ „ other periods: 0·10

The differences from period to period are significant, even if the first period is excluded. It is clear that the low values of 6–12 weeks are in part due to the inclusion of some inert material in the cotyledons, later shed. Whether the high values for 12–18 weeks are a special characteristic of this stage of development, or whether they are fortuitous, associated perhaps with the relationship of harvest dates to 'flushing', cannot be determined without a more extensive series of data. The differences between progenies and their interaction with period are also significant. Though these differences are most probably of genetical origin, that is not necessarily so; only two pods from each parent having been used, it is, for instance, possible that differences in ripeness of these pods might account for them.

The proportional distribution of the increase in dry matter among the various organs of the plant differs substantially from period to period, as is clear from Table IV.

TABLE IV

Mean Percentage Distribution among Organs of Increase in Dry Matter of Whole Plant excluding Cotyledons

	Roots.		Stem.		Leaves of mature 'flushes'.		Leaves of expanding 'flushes'.		Leaves of new 'flushes'.	
Period.	Mean.	S.E. of mean.	Mean.	S.E. of mean.	Mean.	S.E. of mean.	Mean.	S.E. of mean.	Mean.	S.E. of mean.
0–6 weeks	52·6	3·1	9·2	0·3	—	—	—	—	38·2	2·8
6–12 weeks	31·0	1·3	13·5	0·7	7·8	1·6	15·8	3·8	31·9	2·0
12–18 weeks	29·4	1·5	17·4	0·7	3·1	0·6	13·2	3·5	36·9	3·5
18–24 weeks	42·9	2·4	20·8	0·9	3·2	0·6	16·4	2·5	16·7	3·0
24–30 weeks	32·4	3·3	19·4	1·7	1·1	0·6	2·0	0·4	45·1	4·7

In Table IV by 'new flushes' is meant those of which the expansion began after the commencement of the period in question; the terms 'expanding' and 'mature' likewise refer to the condition of the 'flushes' in question at the beginning of the period. The error estimates quoted in Table IV are the root mean square deviations of the percentages for each individual plant from the mean for the progeny of which it is a member; since these individual data are quotients of two variables, and hence will have a non-uniform error component, the error estimates should be taken as no more than a rough indication of the variability of the results.

The principal progressive changes shown in the data are an increase during the first four periods in the proportion of new material laid down in the stem and a decrease in the proportion remaining in the mature leaves. During the fourth period the proportion of new material laid down in the roots was particularly high, that in the leaves of new 'flushes' exceptionally low; the number of new 'flushes' formed during this period was lower than in any other (0·7 per plant), and the high proportionate figure for root increase suggests that root growth may follow a cycle in opposite phase to that of leaf development.

Of the increase in dry weight of the whole plant excluding the cotyledons between 6 and 12 weeks, a substantial part may be accounted for by translocation from the cotyledons, which lost 256 mg. of dry matter between 6 weeks and the time when they were shed. Even if the cotyledons were losing dry matter by respiration during this period at the maximum rate (1 per cent. per day) recorded previously for entire seedlings (Goodall, 1949)—an allowance which seems excessive unless they had at this stage entered a marked climacteric—the amount translocated would still represent 21·4 per cent. of the net gain by the rest of the plant between 6 and 12 weeks, and of course a substantially higher proportion of the gain during the 26 days for which the cotyledons remained attached to the plant.

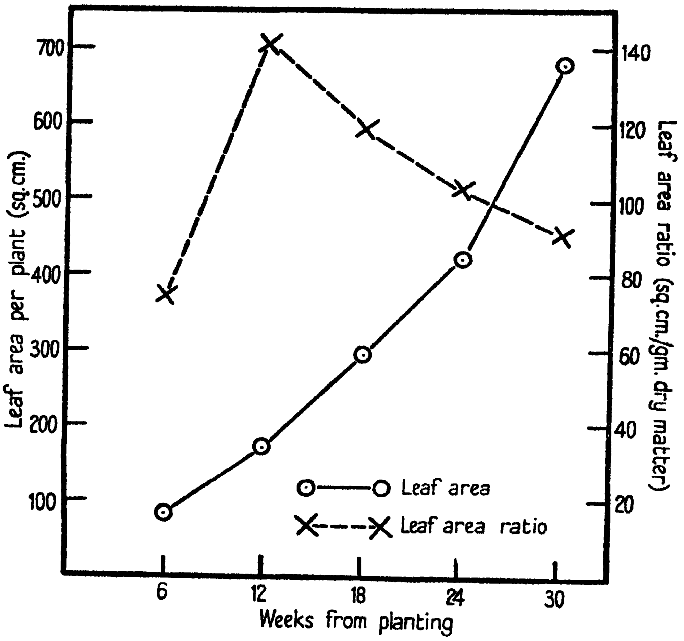

FIG. 2. Changes in the total leaf area per plant and in the ratio of this to the plant dry weight during the first 50 weeks of the development of cacao seedlings.

Leaf area

The development of total leaf area is shown in Fig. 2, sampling errors having been adjusted in the same way as for the dry-weight data in Fig. 1, the 12-week harvest being taken as the basis for adjustment. Table V shows the mean rate of increase in leaf area for plants of each parentage over each period from 6 to 30 weeks. In both Fig. 2 and Table V the area of the cotyledons has been neglected.

TABLE V

Mean Rates of Increase in Leaf Area (sq. cm. per week)

Female parent:	E54/122.	E62/129.	E62/31.	E61/93.	Mean.	S.E. of progeny means.
6–12 weeks	13·4	17·8	17·0	14·1	15·6	1·7
12–18 weeks	13·9	19·0	20·7	24·5	19·5	3·0
18–24 weeks	17·0	19·5	23·1	33·3	23·2	6·6
24–30 weeks	35·3	33·1	39·7	56·9	41·3	10·8
Mean, 6–30 weeks	19·9	22·4	25·1	32·2	24·9	3·3

The rate of growth in leaf area increased progressively during the experiment; the average rate over all periods was significantly greater for the progeny of E61/93 than for the others.

The leaf-area data provide the opportunity for estimating the net assimilation rate of these seedlings. The usual formula for this, as Williams (1946) has pointed out, involves the assumption that changes in leaf area and in plant dry weight are linearly correlated; in a plant like cacao where leaf-area development is irregular it is clear that this assumption is hardly justified. Whereas leaf-area increase in an individual plant follows a step-wise course, that in plant dry matter, apart from diurnal fluctuations, is more nearly continuous, with probably a slight decline in the rate during the periods of most rapid leaf expansion. Consequently some plants might form a new 'flush' and perhaps double their leaf area shortly after the beginning of an experimental period, in which case the usual formula would overestimate the mean net assimilation rate, while in other plants a similar leaf expansion might occur immediately prior to the end of the experimental period and result in an underestimate of the mean net assimilation rate.

The true mean value of the expression $\frac{1}{L}\frac{dW}{dt}$ (L being leaf area, W plant dry weight) over the period studied cannot be found unless the course of increase in both leaf area and plant dry weight is known in detail. In the present work, daily leaf measurements convertible into area estimates were available, but nothing was known of the course of increase in plant dry weight between harvests. In an early paper Gregory (1926) suggested a method of calculating net assimilation rate involving repeated leaf-area measurements and an assumption of linear increase in plant dry weight; it appeared that this assumption would not be far from the truth over a period of 6 weeks in a slowly growing plant like cacao. Accordingly the net assimilation rate for each plant harvested was computed for the preceding period of 6 weeks by two methods: (*a*) the usual formula, including initial and final leaf areas and plant dry weights, involving the assumption of linear relationship between the two variables; and (*b*) Gregory's suggested method, using daily leaf-area estimates with initial and final plant dry weights, involving the assumption of linear increase in plant dry weight. The means computed by both methods are shown in Table VI. Separate estimates having been made for each plant, the standard errors shown have been derived from the variance among these individual estimates. For the period 6–12 weeks, as in determinations of relative growth rate, the dry weight of the cotyledons at abscission had been included in the final plant dry weight.

The error variance of the estimates is greater over the first period (6–12 weeks) than for subsequent periods when computed by method (*a*), but not by method (*b*); furthermore, the error variance is significantly greater using method (*a*) than using method (*b*) up to 18 weeks; both these facts suggest that method (*b*) gives, in this case, more reliable and consistent results than method (*a*). Between 6 and 12 weeks the mean net assimilation rate is

TABLE VI

Mean Net Assimilation Rates of Cacao Seedlings (g./sq. dm./week), computed by Two Methods

Method of computation (see text).	Female parent: Period from planting.	E54/122.	E62/129.	E62/31.	E61/93.	Mean.	S.E. of progeny means.
(*a*)	6–12 weeks	0·0484	0·0798	0·0730	0·0496	0·0627	0·0104
	12–18 weeks	0·0562	0·0830	0·0909	0·0832	0·0783	0·0051
	18–24 weeks	0·0620	0·0691	0·0771	0·0790	0·0718	0·0056
	24–30 weeks	0·0656	0·0709	0·0920	0·1246	0·0883	0·0079
	Mean, 6–30 weeks	0·0581	0·0757	0·0833	0·0841	0·0753	0·0037
(*b*)	6–12 weeks	0·0480	0·0612	0·0593	0·0463	0·0537	0·0058
	12–18 weeks	0·0544	0·0740	0·0799	0·0744	0·0707	0·0037
	18–24 weeks	0·0589	0·0668	0·0784	0·0782	0·0706	0·0048
	24–30 weeks	0·0706	0·0776	0·0962	0·1276	0·0930	0·0081
	Mean, 6–30 weeks	0·0580	0·0699	0·0785	0·0816	0·0720	0·0028

estimated 16·8 per cent. higher by method (*a*) than by method (*b*), and between 12 and 18 weeks 10·4 per cent. higher, while thereafter the differences are negligible. The fact that the discrepancies between the two methods diminish as development proceeds may probably be explained as follows: the proportion in which each new 'flush' increases the pre-existing leaf area of the plant diminishes as the plant grows and the number of 'flushes' it bears becomes greater; consequently, at earlier stages, individual variation in the time of 'flush' expansion within the experimental period would affect the values obtained by method (*a*) more markedly than at later stages.

In the previous study covering the first 7 weeks of growth of cacao seedlings (Goodall, 1949) the maximum net assimilation rate was estimated at 0·090 g./sq. dm./week, and comment was made on the lowness of this value compared with data for other crop plants in the literature. The present results fully bear out this conclusion as to the low net assimilation rate of seedling cacao, but suggest that it was increasing as development proceeded, particularly in the progeny of E61/93. Time and progeny differences and their interaction were all highly significant.

The broken line in Fig. 2 represents the ratio of mean leaf area to mean plant dry weight at each occasion of harvest. It will be noted that this ratio reached a maximum at about 12 weeks, and thereafter declined. The true position of this maximum, had more frequent observations been made, might well have been found to coincide with the time at which expansion of the first 'flush' of leaves was complete, between the 6-week and 12-week harvests. It will be noted, however, that the greatest mean inter-harvest value of this ratio occurred during the period (12–18 weeks) when the greatest relative growth rates were recorded, and may well have been responsible for this.

Water-content

In Fig. 3 the absolute weights of water in the plants and their organs are presented in the same fashion as the dry-matter data in Fig. 1. The diagram speaks for itself, and little comment is necessary. The general picture is very similar to that for dry matter, with the exception of the first 6 weeks after planting, during which the water-content increased by a factor of 9 while the dry weight remained almost unaltered. A detailed study of this period of development has already been published (Goodall, 1949).

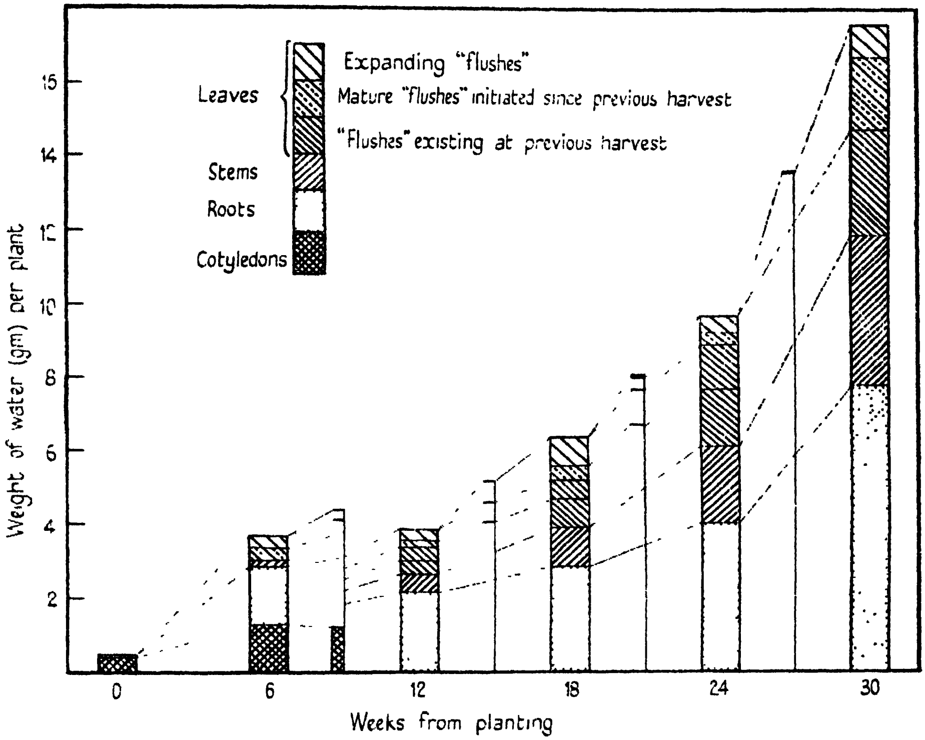

FIG. 3. Increase in weight of water in the cacao seedling and its parts during the first 30 weeks after planting. The narrow intermediate columns represent organs shed during the inter-harvest periods.

In Table VII are shown the proportions in which the net gain in water by the plant as a whole was distributed among the various plant organs; the comments on the standard errors quoted in Table IV are equally applicable here. The cotyledons are not included in Table VII; between planting and the first harvest, the net increase in water-content of the cotyledons amounted to 37·4 per cent. of that in the remainder of the plant, and at 6 weeks they contained 1,282 mg. water per plant. Thereafter there was a decrease in water-content to abscission amounting on average to 62 mg. per plant. By 6 weeks after planting senescence of the cotyledons is already far advanced.

The rate of gain in water-content in the roots as a percentage of that in the

TABLE VII

Mean Percentage Distribution among Organs of Net Increase in Absolute Water-content of Whole Plant excluding Cotyledons

	Roots.		Stem.		Leaves of mature 'flushes'.		Leaves of expanding 'flushes'.		Leaves of new 'flushes'.	
Period.	Mean.	S.E. of mean.	Mean.	S.E. of mean.	Mean.	S.E. of mean.	Mean.	S.E. of mean.	Mean.	S.E. of mean.
0–6 weeks	64·4	2·0	9·9	0·4	—	—	—	—	25·7	1 7
6–12 weeks	41·1	1·7	18·2	1·0	−1·4	0·9	5·7	1·3	36·3	1·7
12–18 weeks	24·7	2·2	23·5	1·7	−4·3	1·9	11·8	3·6	44·3	4·7
18–24 weeks	43·4	4·2	27·3	2·7	−7·0	1·6	14·0	2·7	22·0	3·6
24–30 weeks	49·2	5·0	18·6	5·5	−9·5	2·7	—	—	41·7	4·4

whole plant decreased from germination (at one period of which practically the whole increase in water-content is in the radicle; Goodall, 1949) for about 3 months, and then increased to a value of about 45 per cent. The corresponding figures for the stem increased over the same period, and then remained approximately constant at about 25 per cent. Those for the leaves as a whole increased to a maximum of about 50 per cent. at 3 months, when the leaf-area ratio was at its highest and the relative rate of increase in leaf area also high, and then declined to about 30 per cent. No increases occurred in the weight of water contained in the leaves of mature 'flushes'; indeed, it was decreasing at all times, the rate of decrease in proportion to the increase in the plant as a whole becoming progressively greater as the experiment proceeded and the average age of these leaves increased. The varying distribution of the net gain in water between 'flushes' existing and expanding at the commencement of the 6-week period and those beginning expansion subsequently is probably largely fortuitous, depending upon the coincidence of leaf expansion with time of harvest. For instance, the fact that many 'flushes' were expanding at 18 weeks, few at 24 weeks, and many again at 30 weeks led to a much greater gain in water by new 'flushes' between 24 and 30 weeks than during the previous 6-week period. In the data from which Table VI has been compiled certain significant differences among progenies were found, but they were limited to the first two periods and do not seem to merit further comment.

In Table VIII and Fig. 4 are shown the water-content of the plants and their various organs expressed in terms of dry matter. After the initial rise in the water-content of the plant during the first 6 weeks, it fell steadily to 24 weeks, after which there was a slight but significant rise to 30 weeks. The water-content of roots and stems showed similar changes. Before 24 weeks the water-content of the roots was consistently higher than that of the stems, but in the last two harvests the values for the two organs were very similar. The water-content of the mature 'flushes' was lower than that of the other organs, and declined consistently through the experiment as the average age of the leaves included in them increased; that of the expanding 'flushes' was consistently high, except at 18 weeks, the low value for which may probably be accounted for by

TABLE VIII

Mean Water-content of Plants and Organs, Expressed as Percentage of Dry Weight

Time from planting.	Whole plant.		Roots.		Stem.		Leaves of mature 'flushes'.		Leaves of new 'flushes'.	
	Mean.	S.E. of mean.	Mean.	S.E. of mean.	Mean.	S.E. of mean.	Mean.	S.E. of mean.	Mean.	S.E. of mean.
6 weeks	349	7	559	13	470	10	240	6	376	15
12 weeks	328	8	452	12	359	6	183	4	357	33
18 weeks	266	5	372	9	317	7	172	5	239	9
24 weeks	232	5	276	7	273	8	168	3	410	17
30 weeks	249	5	311	9	307	10	158	6	385	34

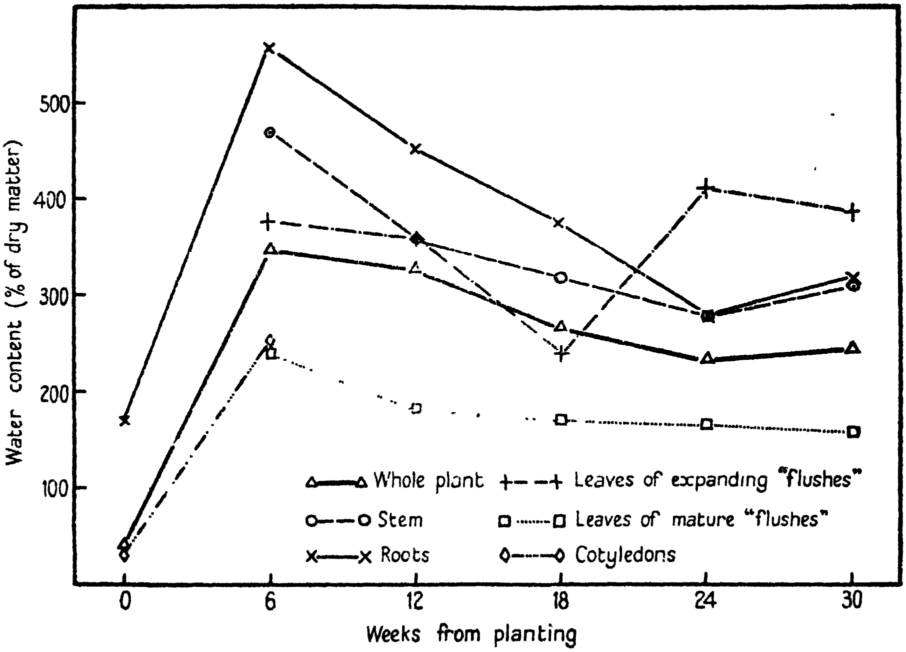

FIG. 4. Changes in the water-content (% of dry matter) of the cacao seedling and its parts during the first 30 weeks of development.

the fact that at this harvest, unlike others, the majority of the leaves in these flushes had completed expansion and were already 'hardening'. The mean percentage water-content of the cotyledons at the first harvest was 256 per cent. (S.E. = 6 per cent.). Parentage affected the percentage water-content of the whole plant, roots, and stem at 30 weeks, and that of the cotyledons at 6 weeks.

In the previous paragraph, attention was drawn to the progressive decline in percentage water-content in the leaves of mature 'flushes'. When data for the individual 'flushes' were separated, a similar decline in each was apparent.

This is shown in Fig. 5 *a*; although leaf expansion had ceased in these 'flushes', the percentage water-content in each continued to decline throughout the experiment, and at all occasions of sampling there was a progressive decrease in water-content from the younger to the older leaves. In the first, third, and fourth 'flushes' the change in percentage water-content during the experiment was adequately accounted for by a similar fall in the absolute water-content (Fig. 5 *b*). In the second 'flush' the mean absolute water-content was less at 12 weeks than at 18 weeks, though thereafter it again decreased; this discrepancy may, however, have been due to the fact that those second 'flushes' which were mature at 12 weeks were probably not a random sample (the smaller 'flushes' maturing earlier), whereas those of plants harvested at later dates were; similar reasoning applies to the fifth 'flush' between 24 and 30 weeks.

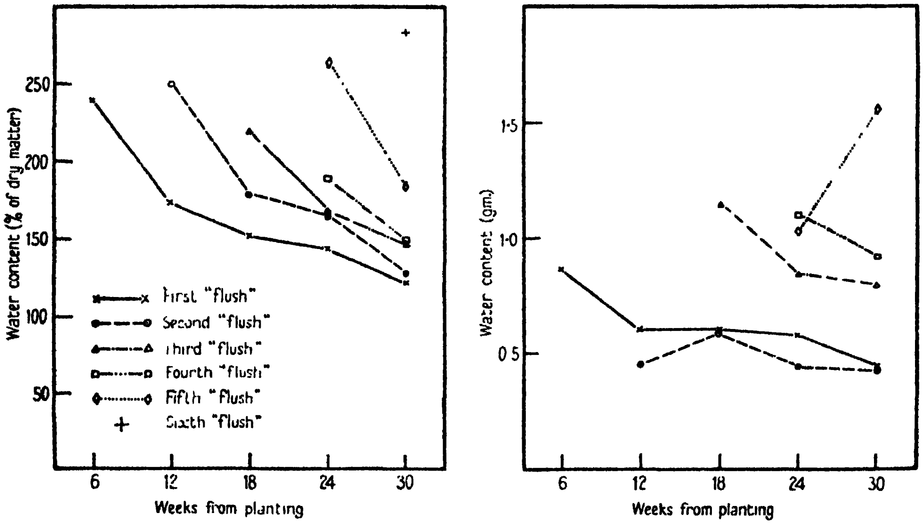

FIG. 5 *a*. FIG. 5 *b*.

FIG. 5 *a* and 5 *b*. Changes in the water-content of successive 'flushes' of mature leaves in the cacao seedling during the first 30 weeks of development, expressed (*a*) as a percentage of dry matter, and (*b*) in grammes.

Water-content data for individual leaves of 'flushes' expanding at the time of harvest are presented in Fig. 6. The relative rate of increase in area (over the period from 48 to 24 hours before harvest—the latest for which information was available) has been taken as an index of the stage of development; this remains constant at about 60 per cent. per day until the leaf reaches half its final area, and then declines sharply—90 per cent. of the final area being attained between 2 and 3 days later. The points in Fig. 6, though very scattered (the error of estimation of the relative rate of leaf-area increase was very high for small leaves), fall clearly around a curve which is asymptotic to a water-content of about 566 per cent. for leaves expanding rapidly, and meets the water-content axis at about 380 per cent. for leaves whose expansion ended one day earlier. It would appear that the leaf water-content begins to fall as soon as the relative rate of leaf expansion decreases, and continues to fall after the leaf, and the 'flush' in which it occurs, have ceased expansion; the mean water-content of fully expanded leaves of 'flushes' of which younger leaves were still expanding was 347 per cent., which may be compared with the values for mature 'flushes' in Table VIII.

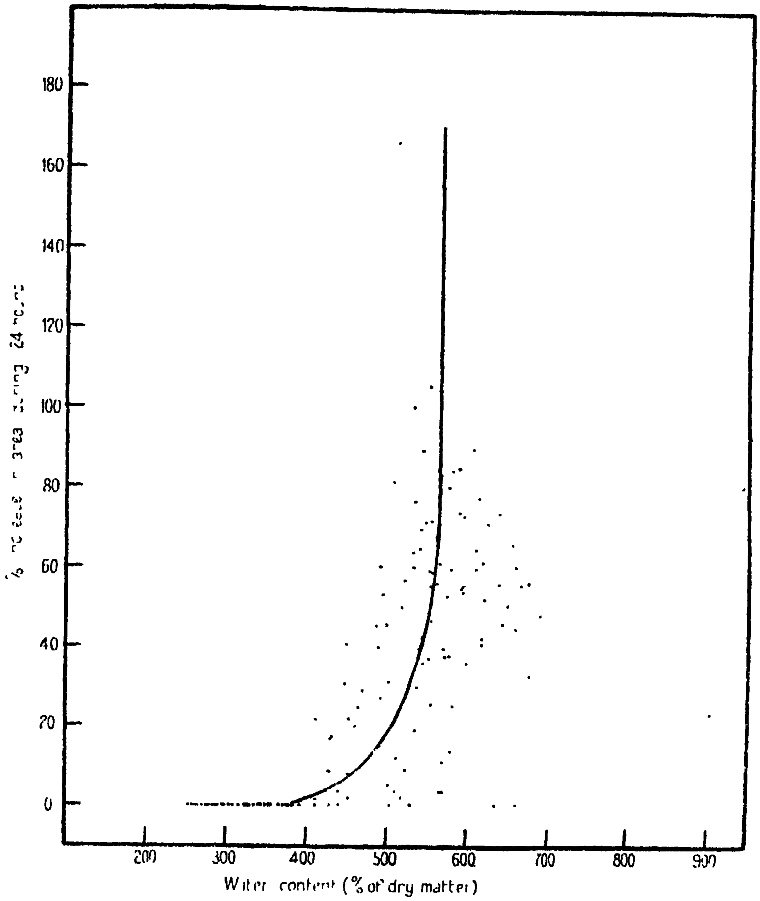

FIG. 6. The relation between the water-content (% of dry matter) of leaves of expanding 'flushes' and their stage of development (as indicated by relative increase in area between 48 and 24 hours prior to harvest).

SUMMARY

The growth of cacao during the first 30 weeks after planting was studied by taking harvests of seedlings at intervals of 6 weeks. The fresh weight and dry weight of each organ individually was determined, and also the area of each leaf. Diagrams are presented showing the mean distribution of dry matter

and water between organs at each harvest. Estimates of changes in weight of the plant and its parts were improved by measuring the intact plants before harvest and correcting for differences in these measurements.

The mean relative growth rate of all plants between 6 weeks and 30 weeks from planting was 1·29 per cent. per day; it varied with the stage of development. The major part of the gain in dry matter occurred in roots and young leaves; the proportion of this gain occurring in the stems increased during the period of the experiment, while that in the older leaves decreased but remained positive.

Though in individual plants growth in leaf area followed a very irregular course, the mean rate showed a progressive increase through the period of the experiment. The net assimilation rate of each plant was computed by the usual method, and by another method (considered preferable in this case) involving daily estimates of leaf area. The mean net assimilation rate between 6 and 30 weeks from planting was 0·072 g./sq. dm./week, but it showed a tendency to increase during the period.

Distribution of water in the seedling agreed in general with that of dry matter, but the older leaves were losing water at a time when they were still gaining in dry matter.

Between 6 weeks and 24 weeks from planting there was a decline in the percentage water-content of the plant and of its various organs, this decline being particularly marked in the roots. The percentage water-content of the young leaves remained nearly constant until half the final area was attained, and then began to decline, this decline continuing for some months after expansion was complete.

Seed from four different female parents was used in the experiment, and it was found that certain of the growth characteristics studied were affected by this factor.

ACKNOWLEDGEMENTS

The author is indebted for criticism of this paper in draft to Mr. O. J. Voelcker, Director of Cacao Research, under whose direction the work was performed, Mr. A. F. Posnette, and Professor J. S. Turner; his thanks are also due to his assistants, Messrs. E. Addo-Yobo, E. N. Afful, P. S. Amfo, A. Bampoe-Addo, J. A. Gordon, J. W. D. Oboo, and J. Quartey for their enthusiastic collaboration.

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The Carbohydrate Nutrition of Tomato Roots

II. The Mechanism of Sucrose Absorption by Excised Roots

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With six Figures in the Text

INTRODUCTION

DORMER and Street (1949) have shown that excised tomato roots are able to utilize sucrose at a much greater rate than either dextrose, laevulose, or an equimolecular mixture of the two and have tentatively advanced the hypothesis that the mechanism of sucrose utilization involves the operation of a specific sucrose phosphorylase at the surface of the absorbing cells. The present investigation was undertaken to test this hypothesis and has involved a study of the effect of selected factors on the carbohydrate uptake of the roots.

GENERAL EXPERIMENTAL PROCEDURE

The general culture technique employed in the establishment and maintenance of our excised root cultures has been previously described (Dormer and Street, 1949). A clone of excised tomato roots (Best-of-All) now in its forty-second passage served as source material for the experimental cultures used in the present investigation.

The stock cultures were maintained in White's synthetic medium (White, 1943) containing 2 per cent. sucrose (S 2% S.). The medium, after autoclaving at 15 lb. for 15 minutes, has a pH of 4·6 to 4·8 (not 4·1 to 4·3 as previously reported in error). There is no evidence that the sucrose suffers any hydrolysis during the autoclaving. Standard culture medium (S 2% S.) prepared either (*a*) by autoclaving the sucrose solution separately and adding it aseptically to a sterile solution of the salts and vitamins, or (*b*) by dissolving solid sucrose aseptically in a sterile solution of the salts and vitamins, has been shown to support root growth qualitatively and quantitatively identical with that in the solution prepared by autoclaving. This contrasts with the work of Burström (1941 *b*), who states that in his excised root culture medium (Burström, 1941 *a*) sucrose is hydrolysed when autoclaved in the presence of the mineral salts.

Stock and experimental cultures were incubated during growth at 27° C. The stock cultures were subcultured every 7 days by White's technique.

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100-ml. wide-necked Hysil flasks containing 50 ml. of culture medium were used in the experiments, and growth was allowed to proceed for 7 days except where otherwise explicitly stated. The methods of expressing results and the calculation of significant differences have been described in a previous paper (Dormer and Street, 1949). The values for mean growth increments (mm.-culture-day) and for dry weights (mg.) are shown in the tables and in the text with their standard errors.

THE pH OF THE CULTURE SOLUTION

1. *The initial pH*

If the mechanism whereby excised tomato roots absorb sucrose involves the operation, at the cell surface, of an enzyme system it is to be expected that the pH of the culture medium will exert a marked effect upon root growth. White (1937), studying the effect of various inorganic nutrient solutions on the growth of excised tomato roots, noted that only those solutions which had a pH below 6·0 were satisfactory. Determining the pH of his own standard medium (50 ml. per culture) after it had supported growth of a root for 7 days, White found that medium of final pH 5·5 was superior to medium of pH 4·5 and markedly superior to media of pH 6·5 or higher. Hitherto no detailed study, with a standard inorganic solution, of the effect of initial pH on the growth of excised tomato roots has been published. Experiments were therefore undertaken to study the effect of the initial pH on the growth of our clone of excised tomato roots.

The pH of the standard culture medium (S 2% S.) was adjusted by the addition of N/20 NaOH or N/20 HCl. The pH adjustment did not cause any precipitation, and it was shown that sodium chloride when added to the standard medium in a concentration to give an excess of sodium or chloride ions over that involved in the pH adjustment was without significant effect on the growth rate. The pH of the medium, after autoclaving, was determined by the glass electrode and the mean value for four flasks, chosen at random, was taken as the pH value. The highest and lowest pH values within the four were required to differ by less than 0·3 pH unit for the batch of medium to be passed.

Experiment No. 1. This covered the pH range 3·65 to 6·75 and had 12 replicate cultures for each pH value.

Experiment No. 2. This covered the pH range 4·3 to 5·8 and had 20 replicate cultures for each pH value.

The results of these two experiments are shown in Fig. 1. These results indicate that good growth is only obtained within the pH range 4·2 to 5·0 with a fairly clearly defined optimum pH at 4·8 to 4·9. At pH values above 5·0 the growth rate falls off rapidly, laterals are inhibited, and the roots and particularly the root-tips become brown. On the acid side of the optimum pH growth is less seriously affected and down to pH 3·9 considerable growth and lateral formation take place. At the pH values 3·65 and 3·9 the roots are thin and translucent, resembling in this respect roots grown with dextrose as their carbon source (Dormer and Street, 1949). Whilst therefore initial pH values above 5·0 have a definitely toxic effect, this is not apparent at the acid end of the pH range tested where the symptoms resemble rather those which appear to be associated with carbohydrate deficiency.

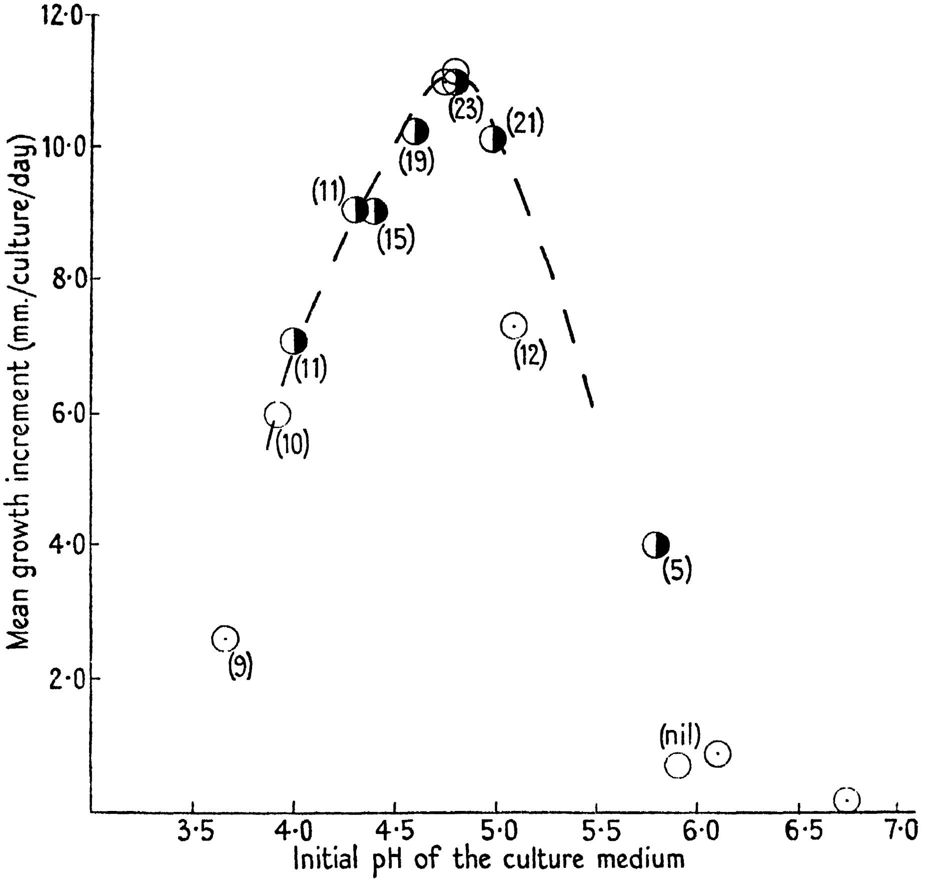

FIG. 1. The effect of initial pH of the culture medium on the growth of excised tomato roots. The mean number of laterals per root is indicated by the number against each mean growth increment value.

The marked pH sensitivity of excised tomato roots stands in contrast to the relative indifference to pH over a fairly wide range of the attached roots. Arnon and Johnson (1942), in a study of the effect of pH on the growth of tomato plants in water culture, reported almost equally good growth over the range pH 4 to pH 8. Excellent and uniform root growth occurred at pH values 5, 6, and 7 and only slightly retarded growth at pH values 4 and 8. Audus (1949), in a study of the growth of seedling cress roots in water culture, observed good growth over the range pH 5·0 to 8·5, with a uniform optimum between pH 5·0 and 6·5.

2. *The pH drift resulting from root growth*

Experiments have been conducted to follow the pH drift which occurs when roots are grown in the standard culture medium of initially favourable pH. Groups of three flasks were withdrawn at intervals during the duration of these experiments and the pH of the medium determined by the glass electrode. The mean pH values obtained in three such experiments are shown in Fig. 2. An alkaline drift in the pH occurs so that 50-ml. stock cultures reach a pH of 5·5 or over by the 7th day. The fall in growth rate which occurs after the 5th day (Fig. 3) is possibly related to this pH drift. This alkaline drift continues for at least 28 days, though the curve clearly flattens out after 14 days growth. Tips taken from cultures 14 or more days old and transferred to medium at pH 4·6 to 4·8 show a lag period of several days, before growth commences and the percentage of tips which fail to grow increases with increasing age of the parent culture. Tips from cultures 14 or more days old when transferred to new medium whose pH has been adjusted to that of the parent culture always fail to grow. There is no evidence of any adaptation of the developing root to growth in medium of alkaline pH, and the pH drift exerts an increasing check to the continuous growth of the cultures.

THE PHOSPHATE NUTRITION OF EXCISED ROOTS

1. *The effect of the level of inorganic phosphate supply on the growth and development of excised roots*

The hypothesis that phosphorylation is involved in sucrose absorption suggested that the level of inorganic phosphate supply might have quite a marked effect on the growth rate.

Experiments Nos. 3 and 4. The standard medium (S 2% S.) contains sodium dihydrogen phosphate ($NaH_2PO_4.2H_2O$) at a concentration corresponding to *c.* 5 p.p.m. P(15 p.p.m. PO_4'''). Media containing various concentrations of this phosphate covering the range 0·0 to 60·0 p.p.m. PO_4''' were prepared. The pH of all media was adjusted to fall within the ranges pH 4·8 to 5·1 for Expt. 3 and pH 4·4 to 4·8 for Expt. 4. Root-tips from the stock cultures were grown for 7 days in the phosphate media, 20 cultures being used for each medium in both experiments. The results are shown in Fig. 4. Growth in length, lateral root formation, and root diameter were found to be indifferent to the phosphate concentration over the range 0·5 to 20·0 p.p.m. Roots growing in the minus-phosphate medium had, however, a significantly lower growth rate, laterals were either absent or one or two in number, and root diameter was reduced as compared with that of roots supplied with phosphate. The growth rate of these minus-phosphate roots declined during the experimental period and the growing apex became thin and delicate and showed a sharp change in direction. The contrast in dry weight between these roots and those grown with phosphate was more marked than the reduction in growth increment value: mean dry weight per root (mg.) for minus-phosphate 0·58±0·11 against 2·15±0·20 for 15·0 p.p.m. PO_4'''.

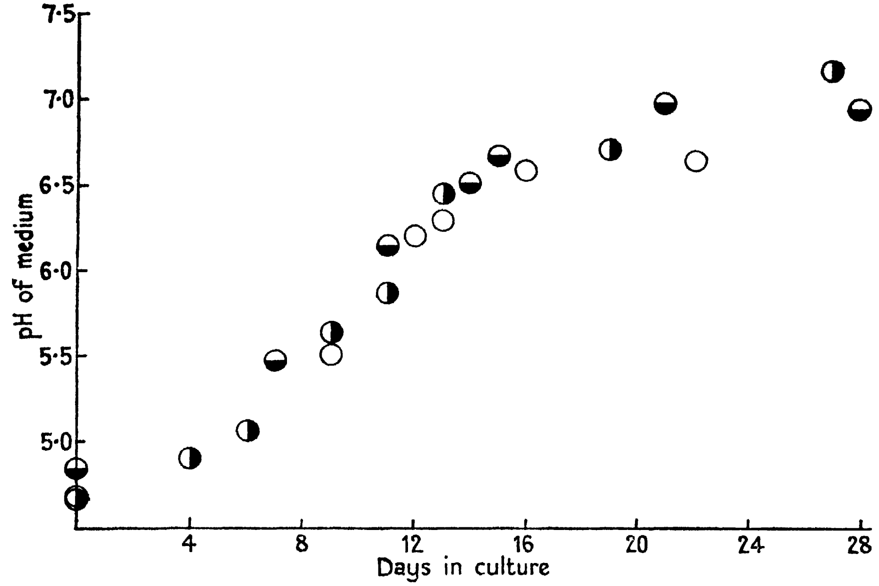

FIG. 2. The alkaline drift in pH occurring as a result of the growth of single root-tip inocula in 50 ml. of sucrose 2 per cent.-synthetic medium.

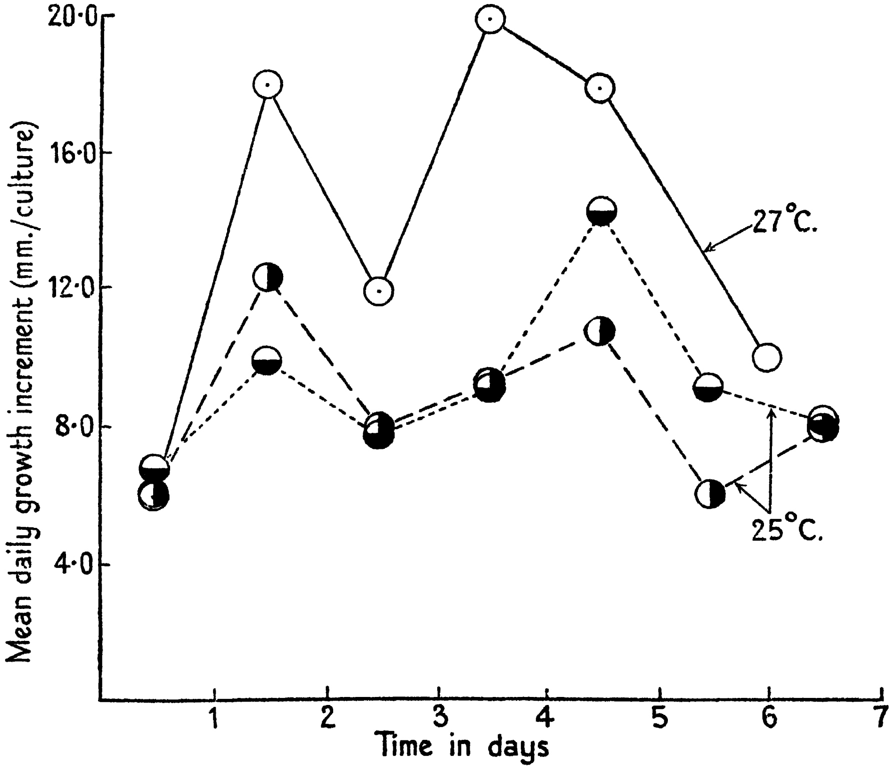

FIG. 3. Mean daily growth increment curves for excised tomato roots showing the decline in growth rate occurring after the fifth day in culture and the temporary check in growth frequently observed on the third day.

Experiment No. 4 a. Since the effect of sub-optimal phosphate concentrations was likely to be masked during the first passage from stock cultures, the roots from Expt. 4 were sub-cultured into phosphate media of the same phosphate concentrations as used for the first passage. A quantitative experiment, involving 20 cultures per phosphate concentration, was then conducted during the 5th passage in the test phosphate media. The results are shown in Fig. 4. The roots grown in minus-phosphate medium could only be taken through a second passage as tip cultures owing to the suppression of lateral root development. During this 2nd passage the growth increment rate fell to 1·9 mm./culture/day (mean of 14 cultures), as compared with a rate of 5·0 mm./culture/day during the 1st passage and tips taken from these 2nd passage cultures failed to grow in an attempted 3rd passage. With the concentration 0·5 p.p.m. phosphate deficiency symptoms absent in the 1st passage became apparent in the 5th passage. The growth increment value fell from 11·2±0·6 mm. in the 1st to 5·4±0·6 mm. in the 5th passage; lateral root development was suppressed and root diameter was reduced. The dry weight data shown in Table I emphasizes this also.

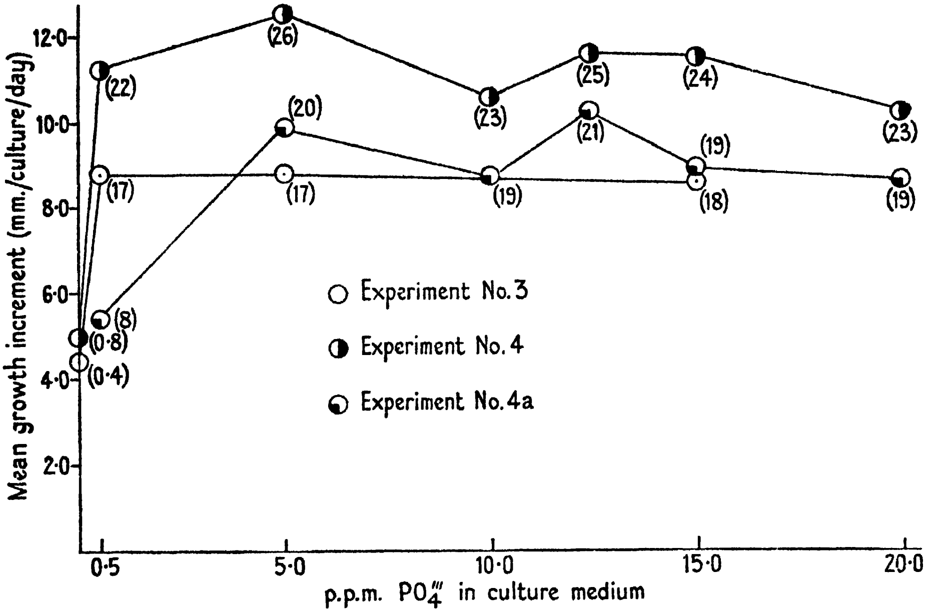

FIG. 4. The effect of the level of the inorganic phosphate supply on the growth of excised tomato roots. The mean number of laterals per root is indicated as in Fig. 1.

Indifference to the phosphate concentration over the range 5·0 to 20·0 p.p.m. is still evident in this 5th passage. It may therefore be concluded that, if phosphorylation is involved in sugar absorption by the excised tomato root, its rate is independent of the external phosphate concentration (except when phosphate starvation is induced), suggesting that a phosphate cycle, not directly influenced by the external phosphate concentration, must be operative within the cell.

TABLE I

(*Experiment 4a*)

p.p.m. PO_4'''	Mean dry wt. per root (mg.).
0·5	0·75±0·05
5·0	1·95±0·23
10·0	1·55±0·12
12·5	1·80±0·14
15·0	1·50±0·13
20·0	1·50±0·24

2. *The respiratory behaviour of phosphate-deficient excised roots*

Saïd (1948) reports, without giving actual experimental data:

(*a*) that the cut ends of leaves dipped in sucrose solution hydrolyse the sugar before absorbing it;

(*b*) that the increase in respiration rate resulting from sucrose feeding is reduced when the leaves are phosphorus-deficient and that this is associated with a reduced rate of inversion of the sucrose and a slower rate of uptake of the hydrolysis products;

(*c*) that feeding with neutral phosphate and sucrose together restores the normal hydrolytic and absorptive capacities of the phosphorus-deficient leaves.

On the basis of these observations, Saïd has advanced the hypothesis that phosphorus deficiency in leaves leads to reduced 'invertase' activity at the cell surfaces. An experiment was therefore undertaken to see if a similar behaviour characterized phosphorus-deficient excised tomato roots.

Experiment No. 5. Oxygen uptake of root sectors was determined by the direct method of Warburg. The Warburg flasks used were fitted with a centre well and single side arm. The centre well was charged with 0·2 ml. 10 per cent. w/v sodium hydroxide by the standard method. The body of the flask contained 2 ml. of S 0% S. minus phosphate solution to suspend the root sectors. The side arm contained 0·5-ml. treatment solution. 12 to 16-mm. root sectors were cut from the terminal 35 mm. of roots grown respectively in the standard S 2% S. (15 p.p.m. phosphate) medium and S 2% S. minus phosphate medium. The sectors were washed by transference to two changes of sterile S 0% S. minus phosphate solution and after 1 hour were transferred to the Warburg flasks. Each flask contained either 8×S 2% S. (15 p.p.m. phosphate) sectors or 12×S 2% S. minus phosphate sectors. The flasks were then fitted to their manometers and transferred to the bath at 27° C. and allowed to equilibrate for 15 minutes. The initial O_2 uptake per hour was determined over the period 0 to 2 hrs. The treatment solution was then tipped in from the side arm and the average O_2 uptake per hour determined over the periods 2 to 6 hrs., 6 to 18 hrs., and 18 to 22 hrs. The rates of o_2 uptake after addition of the treatment solutions are expressed as percentages of the initial rates in Table II. Each value in this table is the mean of six separate runs, performed with separate batches of root material. There were no signs of bacterial contaminations in any of the flasks at the end of the runs and the thermobarometer, which always contained S 2% S.+15 p.p.m. phosphate, never showed any significant uptake of oxygen. The S 0% S. minus phosphate solution used was sterile and the treatment solutions were freshly prepared with fresh double-distilled water and were stored in the refrigerator. Sectors from roots grown on S 2% S. (15 p.p.m. phosphate) medium when suspended in S 0% S. (15 p.p.m. phosphate) solution showed a fall in the rate of o_2 uptake during the period 2 to 6 hours to give a rate, subsequently maintained during the whole experiment and corresponding to 60 to 65 per cent. of the initial rate. When these sectors were supplied with sucrose either in the absence or presence of external phosphate a steady rise in the o_2 uptake took place until by the end of the experimental period the rate was 200–250 per cent. of the initial rate.

TABLE II

Experiment No. 5: Oxygen Uptake of Root Sectors obtained from Excised Tomato Roots (Suspending medium S 0% S. minus phosphate.)

Root sectors from	Experimental treatment (commencing at 2 hrs.)	Mean values for oxygen uptake per hour during treatment expressed as a percentage of oxygen uptake per hour during the period 0 to 2 hrs.		
		2 to 6 hrs.	6 to 18 hours	18 to 22 hrs.
Normal roots (grown on S 2% S.+15 p.p.m. PO_4''')	15 p.p.m. PO_4'''	65	64	60
	Sucrose 2%	112	141	201
	15 p.p.m. PO_4'''+ +Sucrose 2%	83	117	256
Phosphorus-deficient roots (grown on S 2% S. minus PO_4''')	15 p.p.m. PO_4'''	105	99·5	123
	Sucrose 2%	104	95	86
	15 p.p.m. PO_4'''+ Sucrose 2%	127	188	457

Sectors from roots grown on S 2% S. (minus phosphate) medium had a low rate of respiration which was, however, well maintained when the sectors were suspended in S 0% S. (15 p.p.m. phosphate) solution. When supplied with sucrose in the absence of external phosphate a slow decrease in the o_2 uptake of the sectors took place, but when supplied with sucrose and phosphate simultaneously a rapid increase in the rate of o_2 uptake occurred so that by the end of the experimental period the rate achieved was some 450 per cent. of the initial rate. The phosphorus-deficient root sectors closely paralleled in respiratory behaviour the phosphorus-deficient leaves used by Saïd.

The rapid restoration of the sucrose absorbing ability of both phosphorus-deficient leaves and roots resulting from an external supply of inorganic phosphate strongly suggests a phosphate activation of the absorbing mechanism rather than a specific role for phosphate in the synthesis of the enzyme(s) involved in sugar utilization. The absence of any direct evidence for an activation of 'invertase' by phosphate therefore also from the invertase hypothesis and enhances the view now postulated that a phosphorolysis of sucrose is involved in sugar absorption.

THE PHLORIDZIN INHIBITION OF ROOT GROWTH

Lundsgaard (1933) and Kalckar (1937) suggested that phloridzin prevents glucose reabsorption in the proximal tubules of the kidney by inhibiting the phosphorylation of glucose. Ostern *et al.* (1936) showed that phloridzin prevents the formation of glucose-6-phosphate in muscle extracts incubated with glycogen and inorganic phosphate. Dahl (1939) obtained similar results. Cori, Colowick, and Cori (1939) and Cori and Cori (1940) studying the kinetics of glycogen synthesis from glucose-1-phosphate reported phloridzin and phloretin to be inhibitors of glucosan phosphorylase. Phloridzin at 0·01 M caused 85 per cent. or greater inhibition of phosphorylase activity. Shapiro (1947), using minced rat kidney tissue, found certain dehydrogenases to be much more sensitive to phloridzin poisoning than the glucose phosphorylating system, though the latter was reported to be significantly inhibited by 0·001 to 0·003 M phloridzin.

Lisitsyn (1941) is reported by Hartt (1943) to have found that 0·005 M phloridzin inhibited the synthesis of sucrose in the leaves of *Arctium lappa* and *Crataegus* sp. but not in the leaves of *Tussilago farfara*. Kriukova (1940), however, failed to detect inhibition of sucrose synthesis in leaves infiltered with phloridzin, and Hartt (1943), without giving experimental details, reported that phloridzin at 0·01 M concentration did not interfere with sucrose synthesis in detached leaf blades of sugar-cane. Little significance can, however, be attached to these negative results as there is no evidence that, with the experimental conditions employed, the phloridzin was brought into contact with the sucrose synthesizing system. Doudoroff (1943) has, however, shown that phloridzin, at concentrations between M/400 and M/150, is without significant effect on the activity of bacterial sucrose phosphorylase. Whilst therefore phloridzin inhibits certain phosphorylations, there is no reliable data concerning its effect on sucrose phosphorolysis or synthesis in higher plants. It was, however, considered desirable to study the action of phloridzin on the utilization of sucrose by excised tomato roots in view of its general ability to inhibit phosphorylating systems.

1. *Purification of phloridzin*

The commercial phloridzin available was a very pale yellow crystalline powder with a solubility of about 1 g. in 1,000 g. water at 20° C. The saturated aqueous solution was almost colourless when prepared but gradually turned bright yellow on storage, particularly when kept on the alkaline side of neutrality or exposed to light. The standard culture medium containing 0·1 per cent. w/v of this commercial phloridzin turned bright yellow on autoclaving at 10 lb. for 15 minutes. No precipitation of phloretin (insoluble in water) or significant change in pH occurred, and it was therefore concluded that the coloration was not associated with hydrolysis of the glucoside but was probably due to the presence of impurity. When commercial phloridzin was purified by the procedure described below, which is a modification of that used by Moelwyn-Hughes (1928), it was obtained as colourless needles. These dissolved in water to give a colourless solution which developed little or no coloration on autoclaving and only turned pale yellow after 7 days' incubation at 27° C.

Procedure. 15 g. of commercial phloridzin were dissolved in 1 litre of distilled water at 60° C. and the solution set aside in the refrigerator at 4° C. overnight. The crystals were collected and redissolved in 1 litre of distilled water. The solution was heated in a water-bath to 75° C. to 80° C. and treated for 10 minutes with 10 g. of activated charcoal. The solution was filtered and set aside to crystallize overnight in the refrigerator. The crystals were collected and recrystallized a third time from 1 litre of distilled water. The crystals were collected on a No. 50 Whatman filter and dried in a desiccator over phosphorus pentoxide. This purified phloridzin was used in the experiments described below.

2. *Effect of phloridzin concentration on the growth of excised root supplied with 2 per cent. sucrose*

Experiment No. 6. Phloridzin was added to the standard culture medium (S 2% S.) to give the following concentrations: 0·1 (0·0021 M), 0·05, and 0·01 per cent. The media were sterilized by autoclaving at 10 lb. for 15 minutes. The experiment involved 40 cultures; 10 replicates of each medium being inoculated.

Experiment No. 7. S 2% S. medium containing the following concentrations of phloridzin was employed: 0·1, 0·07, 0·05, 0·03, and 0·01 per cent. The experiment involved 120 cultures; 20 replicates of each medium being inoculated.

The results of these experiments are shown in Table III. Phloridzin inhibited growth in length and lateral root formation and at the higher concentrations caused a reduction in root diameter. The inhibitory effect on both growth in length and lateral formation increased regularly with increasing concentration of phloridzin up to the highest concentration used (0·1%). The absence of any obvious toxic symptoms supported the view that phloridzin interferes with the carbohydrate nutrition of the excised root. The inhibitory effect of phloridzin on growth in length is shown by calculation of the 'percentage inhibition' from the equation:

$$\text{Percentage inhibition} = \frac{(\text{S } 2\%\ \text{S.})-(\text{S } 2\%\ \text{S. Phloridzin})}{(\text{S } 2\%\ \text{S.})}\times 100,$$

where (S 2% S.) and (S 2% S. phloridzin) are the mean growth increments in mm.-culture-day respectively of the standard medium (control cultures) and the standard medium containing the test concentration of phloridzin.

TABLE III

The Effect of Phloridzin on Excised Roots growing in Synthetic Medium containing 2 per cent. Sucrose

Experiment No.	Character measured	Percentage phloridzin. 0·0 control.	0·01	0·03	0·05	0·07	0·10
6	Mean percentage inhibition of growth in length . .	—	22·3	—	48·6	—	73·4
7	Mean percentage inhibition of growth in length . .	—	26·0	42·0	55·8	58·6	67·3
	Mean number of laterals per root .	23	18	10	6	5	1

3. *Effect of the concentration of sucrose on the inhibition of growth caused by phloridzin*

Experiment No. 8. The standard culture medium containing 1, 2, and 3 per cent. sucrose was used. Half the cultures contained 0·04 per cent. phloridzin. The experiment involved 120 cultures; 20 replicates of each medium being inoculated. The results are shown in Table IV. It is clear that the degree of inhibition decreases with increasing concentration of sucrose, strongly suggesting competition between sucrose and phloridzin for an enzyme system essential for the carbohydrate nutrition of the excised root.

TABLE IV

The Effect of the Concentration of Sucrose on the Inhibition of Excised Root Growth caused by 0·04 per cent. Phloridzin (Data from Experiment No. 8)

Character measured.	Percentage sucrose. 1·0	2·0	3·0
Mean percentage inhibition of growth in length caused by 0·04 per cent. phloridzin . .	64·3	43·0	25·1
Mean number of laterals per root in the controls	12	19	10
Mean number of laterals per root in presence of 0·04 per cent. phloridzin	0	10	10

4. *The reversibility of phloridzin inhibition*

Experiment No. 9. Two groups (*A* and *A′*) of twenty 4-day-old cultures in sucrose 2 per cent. synthetic medium and two similar groups (*B* and *B′*) in sucrose 2 per cent. synthetic–phloridzin 0·05 per cent. medium were measured and the mean growth increment of each group calculated. The roots from the first group (*A*) in S 2% S. medium were then transferred aseptically to S 2% S. medium previously brought to 27° C. in the incubator. The roots from the second group (*A′*) were then transferred aseptically to new S 2% S. phloridzin 0·05 per cent. medium also at 27° C. The roots from the first group (*B*) in S 2% S. phloridzin 0·05 per cent. medium were transferred to new S 2% S. phloridzin 0·05 per cent. medium, and the roots from the second group (*B′*) were transferred to new S 2% S. medium by the same technique. All cultures were measured and the laterals counted at the end of the 3rd day following transference (168 hours from the initiation of the cultures). The results are summarized in Table V. It is clear that an initial period of 4 days in the standard medium mitigates the effect of phloridzin and eliminates the decrease in lateral root formation normally associated with phloridzin inhibition. A 4-day period of moderate phloridzin inhibition does not prevent a subsequent increase in growth rate when the roots are transferred to phloridzin free medium.

TABLE V

Reversibility of the Phloridzin Inhibition of Excised Root Growth
(Data from Experiment No. 9)

0 to 96 hours		96 to 168 hours		
Culture medium.	Mean value mm./culture/day during period.	Mean value mm./culture/day during period.	Mean number of laterals per root at 168 hours.	Culture medium.
Sucrose 2%-synthetic (*A*)	6·1 ± 0·7	11·1 ± 1·9	15	Sucrose 2%-synthetic
Sucrose 2%-synthetic (*A′*)	6·8 ± 0·6	6·6 ± 0·6	15	Sucrose 2%-synthetic + 0·05% phloridzin
Sucrose 2%-synthetic + 0·05% phloridzin (*B*)	3·5 ± 0·3	3·5 ± 0·3	3	Sucrose 2%-synthetic + 0·05% phloridzin
Sucrose 2%-synthetic + 0·05% phloridzin (*B′*)	3·6 ± 0·3	5·2 ± 0·4	5·5	Sucrose 2%-synthetic

Experiment No. 10. The roots of three groups of twenty-two 2-day-old cultures in S 2% S. medium were measured. Using the technique described in Expt. 9, the roots of one group were transferred to new S 2% S. medium, and the roots of the other two groups transferred to S 2% S. phloridzin 0·1 per cent. medium. After 48 hours the roots were measured and the mean growth increment for each group determined. The roots in S 2% S. medium were then transferred to new S 2% S. medium and one group of the roots in S 2% S. phloridzin 0·1 per cent. medium transferred to new S 2% S. phloridzin 0·1 per cent. medium. The second group of roots in S 2% S. phloridzin 0·1 per cent. medium were transferred to new S 2% S. medium. After 48 hours the roots were again measured and the laterals counted. The results are summarized in Table VI. The 2-day period in S 2% S. medium protects the roots from the full inhibitory effect of phloridzin, and roots inhibited by a concentration of phloridzin as high as 0·1 per cent. quickly show an increase in growth rate when transferred to phloridzin-free medium. The 2-day period in S 2% S. medium is also sufficient to reduce the inhibitory effect of phloridzin on lateral root formation and the development of laterals increases significantly if the 2-day phloridzin treatment is followed by removal to phloridzin free medium.

Mean growth increments for each separate 24 hours, during the 7-day growth period in S 2% S. medium, are shown in Fig. 3. The data from

TABLE VI

Reversibility of the Phloridzin Inhibition of Excised Root Growth (Data from Experiment No. 10)

48 to 96 hours		96 to 144 hours		
Culture medium.	Mean value mm./culture/day during period.	Mean value mm./culture/day during period.	Mean number of laterals per root at 144 hours	Culture medium.
Sucrose 2%-synthetic	13·1±1·1	18·0±1·4	19	Sucrose 2%-synthetic
Sucrose 2%-synthetic+0·10% phloridzin	4·9±0·4	5·6±0·4	6	Sucrose 2%-synthetic+0·1% phloridzin
Sucrose 2%-synthetic+0·10% phloridzin	4·5±0·3	11·2±0·8	11	Sucrose 2%-synthetic

Expts. 9 and 10 suggests that lateral initiation takes place early in the growth period and that many of the laterals visible after 7 days are formed during the first 96 hours. The significant decrease in growth increment rate frequently noted on the third day of growth in S 2% S. medium (Fig. 3) is therefore probably related to the diversion of food material to lateral root meristems. This early lateral root formation would explain why phloridzin treatment subsequent to 48 hours fails to suppress completely the development of laterals and if applied after 96 hours fails even to reduce their number (Expt. 9).

5. *Effect of phloridzin on the growth of seedling roots*

The reversibility of phloridzin inhibition, its proportionality to phloridzin concentration, and the ability of increased external sucrose concentration to reduce significantly the effect of the phloridzin strongly suggest that this substance acts as a competitive inhibitor of sucrose absorption. The possibility remained, however, that phloridzin penetrates into the cells in significant amounts, inhibits growth by interference with the general cell metabolism, and that the reversibility of phloridzin inhibition is due to operation of a detoxicating mechanism. Such an hypothesis, postulating a general effect on cell metabolism rather than a specific surface effect on the enzyme system mediating sucrose absorption, would be supported if the seedling root receiving as it does an internal sugar supply from the seed should show a similar phloridzin inhibition to the excised root.

Experiment No. 11. Sterile seedling cultures were set up by the technique previously described (Dormer and Street, 1949), using as media S 0% S. and S 0% S.+0·07% phloridzin. The seedlings (35 per treatment) were harvested 8 days after surface sterilization of the seed and the length of each radicle measured. The results obtained for the mean radicle lengths (in mm.) were: Control S 0% S. = 39·0±1·61; 0·07% phloridzin = 42·0±1·85. No significant difference in length was found. In general appearance the seedlings grown in the control and in the phloridzin medium were indistinguishable. The rather large standard error recorded for these seedling roots is due to the variable time of germination within each treatment. Excised roots growing in sucrose medium when treated with the same concentration of phloridzin suffer a 58 per cent. inhibition of their growth in length. The absence of any inhibition of the growth of the seedling roots is considered to support strongly the view that phloridzin inhibition of the excised roots results from a retardation of sucrose absorption rather than from any toxic action of the glucoside on general cell metabolism.

THE EFFECT OF VARIOUS SUGARS ON THE GROWTH OF EXCISED ROOTS SUPPLIED WITH SUCROSE

Doudoroff (1943) has shown that dextrose inhibits the action of the sucrose phosphorylase of *Pseudomonas saccharophila*. Later Doudoroff, Barker, and Hassid (1947) found that xylose, arabinose, and galactose have a much weaker inhibitory action on this bacterial sucrose phosphorylase than does dextrose.

1. *The inability of various sugars to support the growth of excised roots*

The habit and growth rate of excised tomato roots supplied with dextrose as the sole carbon source has been described in a previous paper (Dormer and Street, 1949). A number of other sugars have been tested in our laboratory for their ability to support the growth of excised tomato roots. Galactose (2 samples), mannose (2 samples), arabinose, xylose, rhamnose, and maltose (2 samples), all used at 2 per cent. concentration in the standard synthetic medium, proved unable to support growth: the standard 10-mm. root-tip inocula when supplied with these sugars shows a total extension of less than 5 mm. in 7 days. Two samples of laevulose failed to support growth, whilst a third sample supported a mean daily extension of only 1·4 mm. for 5 days, after which growth ceased. This extension was associated with a decrease in root diameter and a curling of the tip (a feature frequently noted in dextrose grown roots). Raffinose (2 samples), used at 3 per cent. concentration, supported a rate of extension ranging from 0·7 to 1·0 mm. per day, growth frequently ceasing before completion of the 7-day incubation period. Laevulose and raffinose are clearly unable to support even the slow but maintained rate of growth obtained with dextrose media. The other sugars tested as alternatives to sucrose have proved therefore, with the exception of dextrose, unable to support the growth of excised tomato roots. The experiments described below were undertaken to study the effect of these sugars on the growth of excised tomato roots simultaneously supplied with sucrose.

2. *The inhibitory effect of various sugars on the growth of excised roots supplied with sucrose*

Experiment No. 12. This experiment involved 7 culture media and 20 replicate cultures for each medium. All completed media were sterilized by autoclaving at 10 lb. for 15 minutes. A preliminary experiment had shown that growth in raffinose-containing media was not significantly affected by autoclaving the sugar and salts separately as against autoclaving the complete media. The root-tips were grown in the experimental media for 5 days. The results are shown in Table VII. Galactose, maltose, xylose, and laevulose, at the concentration used, almost completely inhibited the growth of roots simultaneously supplied with 1 per cent. sucrose. White (1940) has found galactose, maltose, and laevulose unable to support the growth of excised tomato roots and described definite toxic symptoms developed in their presence. Burström (1948) has reported that galactose is not only unable to support the growth of excised wheat roots but has a specific toxic action. Wheat roots readily utilized dextrose as a carbon source, but the addition of galactose to the dextrose medium not only inhibited root growth but caused rapid death of the meristem. The inability of excised tomato roots to grow in presence of galactose, maltose, xylose, and laevulose probably results from a similar toxic action rather than from a marked inhibition of the sucrose absorbing mechanism by these sugars. The accumulation of laevulose during the growth of excised tomato roots supplied with sucrose (Dormer and Street, 1949) would appear to be an important 'staling' factor. Raffinose exerted no deleterious effect upon growth and it was noted that its presence was associated with a more even growth of the replicate cultures than occurred in the S 1% S. medium.

TABLE VII

The Effect of Various Sugars on the Growth of Excised Tomato Roots simultaneously supplied with 1 per cent. Sucrose. Growth Period 5 Days. (Data from Experiment No. 12)

Culture medium.	Initial pH.	Mean growth increment mm./culture/day.	Mean number of laterals per root.
S 1% S.	4·6	10·1±1·3	12
S 1% S.+1% Dextrose	4·3	5·3±0·6	9
S 1% S.+1% Laevulose	4·3	1·3±0·2	1
S 1% S.+1% Galactose	4·3	0·30±0·04	—
S 1% S.+1% Xylose	4·5	1·5 ±0·3	0·2
S 1% S.+2% Maltose	4·2	0·9 ±0·2	1
S 1% S.+3% Raffinose	4·7	11·9 ±0·7	15

3. *Dextrose as an inhibitor of sucrose utilization*

Experiment No. 13. This experiment was designed to study the effect of sucrose concentration on root growth and to see how far the inhibitory effect of 1 per cent. dextrose would be dependent upon the concentration of sucrose simultaneously supplied. It involved 10 culture media and 20 replicate cultures for each medium. The media were adjusted in pH so that, after autoclaving, the extreme limits of initial pH for the experiment were 4·5 to 5·0. The small pH differences between the experimental media did not obscure the effects on root growth of sucrose concentration and of the presence of dextrose. The results are summarized in Fig. 5.

Optimum growth was obtained with 2 per cent. sucrose. With 0·1 per cent. and 0·5 per cent. sucrose the roots resembled those grown in dextrose media showing the reduction in root diameter and absence of laterals. The deleterious effect of high sucrose concentration, previously observed with 4 per cent. sucrose (Dormer and Street, 1949), is noteworthy. At 5 per cent. concentration a very high percentage of the roots are 'sinkers' (White, 1943, p. 140). This enhanced specific gravity, as it is associated with thickening of the root axis, suggests carbohydrate accumulation rather than an inhibition of air-space development.

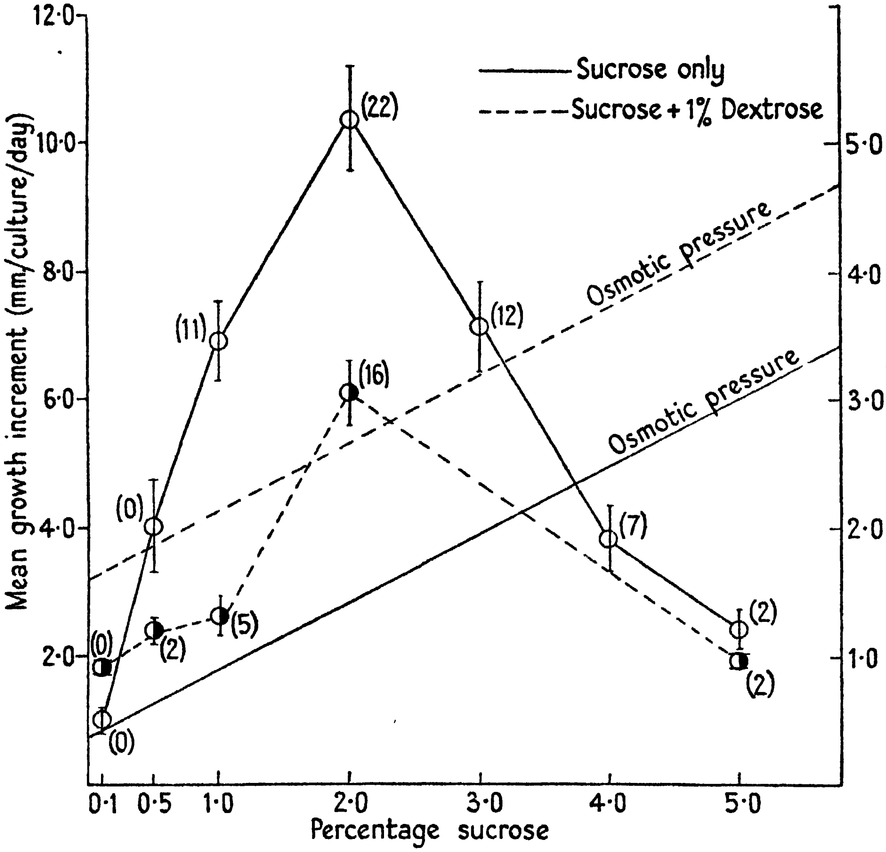

FIG. 5. The effects on the growth of excised tomato roots of the sucrose concentration and of the addition of dextrose to sucrose-containing media. The lengths of the vertical black lines represent twice the standard errors of the mean growth increment values plotted with the mean as a mid-point. The mean number of laterals per root is indicated as in Fig. 1.

The addition of 1 per cent. dextrose to solutions containing 0·5, 1·0, and 2·0 per cent. sucrose caused a decrease in root growth and with 1·0 and 2·0 per cent. sucrose a decrease in the mean number of laterals per root. The proportional decrease in growth rate caused by 1 per cent. dextrose in presence of 1 per cent. sucrose was more marked than in presence of 2 per cent. sucrose, suggesting that dextrose causes a competitive inhibition of sucrose utilization. The presence of dextrose was not associated with toxic symptoms and with 0·1 per cent. sucrose the addition of 1 per cent. dextrose actually raised the growth rate slightly, the roots being identical in appearance with dextrose-grown roots, suggesting that dextrose had displaced sucrose as the main carbon source.

4. *The effect of sucrose concentration and osmotic pressure on root growth*

Experiment No. 14. This experiment was designed to study the effect of 3 per cent. raffinose on the growth of roots supplied with various concentrations of sucrose and the effect of sucrose concentration on root growth when all solutions are adjusted to the same osmotic pressure by raffinose. It involved 11 culture media and 19 replicate cultures for each medium. After autoclaving the initial pH values of the media fell within the limits pH 4·55 to 4·85, with the exception of S 0·5% S.+3 per cent. raffinose medium which had an initial pH of 5·1. The results are summarized in Fig. 6.

This experiment confirmed that with the standard synthetic medium 2 per cent. sucrose is superior to the other sucrose concentrations tested and that raffinose does not cause any retardation of the growth of excised tomato roots simultaneously supplied with sucrose.

In both Expts. 13 and 14 it was observed that the roots grown with 1·0 per cent. or less sucrose were delicate in structure and that with 0·5 per cent. or less sucrose they are clearly limited in growth by deficiency of carbohydrate. Roots receiving 3 per cent. or more sucrose were characterized by an increase in diameter and yellowing of the main axis as compared with roots grown in the standard medium (S 2% S.), and by a reduction in the length of the laterals. At 4 per cent. and 5 per cent. sucrose concentration the laterals were reduced in number and were distorted. At 4 per cent. sucrose some 40 per cent. and at 5 per cent. some 80 per cent. of the roots examined were 'sinkers'.

By using raffinose (Expt. 14) to adjust the osmotic pressure of media containing 1, 2, and 3 per cent. sucrose so that they were equal in O.P. to S 4% S. medium it became evident that the deleterious effect of 4 per cent. sucrose is not due to the enhanced O.P. of the medium but to the concentration of sucrose itself. This combined with the data presented above suggests that high sucrose concentrations are deleterious to root growth because they derange the normal carbohydrate metabolism of the roots.

The addition of raffinose to S 1% S. medium enhanced the growth rate and uniformity of development assessed after 7 days' incubation. This effect, presumably due to increase in the osmotic pressure of the medium, is noticeable even after only 5 days' incubation (Expt. 12). The inhibition of growth caused by the addition of 1 per cent. dextrose to S 1% S. medium is therefore very marked compared with that occurring in presence of 2 per cent. sucrose for the dextrose in enhancing the O.P. of the S 1% S. medium should be beneficial to root growth. The inability of raffinose to enhance the growth of roots supplied 0·5 per cent. sucrose confirmed that raffinose is unable to act as a carbon source for the growth of excised tomato roots and that at this sucrose concentration the extent of root growth is controlled by a deficiency of utilizable carbohydrate.

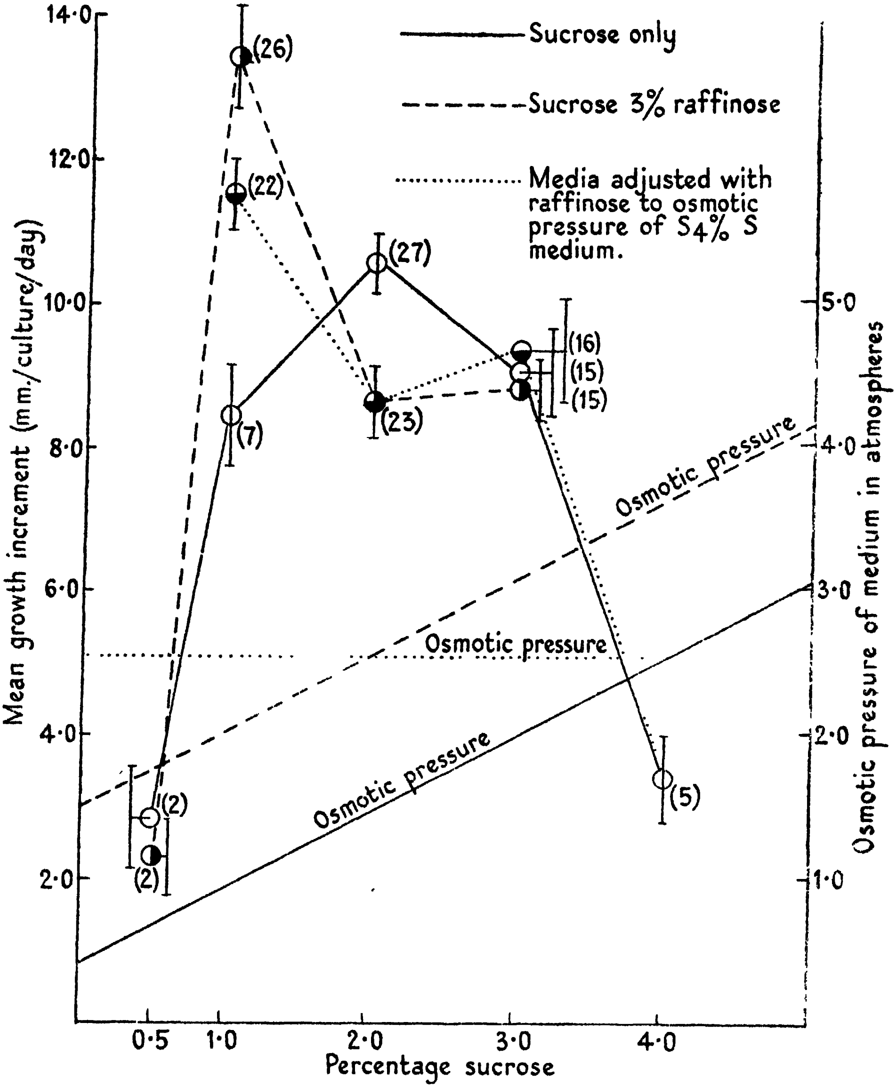

Fig. 6. The effects on the growth of excised tomato roots of the sucrose concentration and of the addition of raffinose to sucrose-containing media. Standard errors are indicated as in Fig. 5 and the mean number of laterals per root as in Fig. 1.

DISCUSSION

Recent studies of cell permeability have supported the hypothesis that there exists at the protoplast surface a bi-molecular layer of lipoidal units which is overlaid with unfolded protein units orientated tangentially with the surface (Davson and Danielli, 1943). This surface layer represents the main energy barrier to the entry of solutes into the cytoplasm and leads to the conception that entry involves penetration of the lipoid-protein film, passage through the film, and liberation into the cytoplasm. Such a film as that postulated will offer a marked resistance to the intake of highly polar molecules such as sugars.

The high content of protein in the surface film and its undoubted metabolic activity, as evidenced by cell-wall formation, makes it possible that a number of enzymes and particularly those involved in carbohydrate synthesis and degradation may be located in the protoplast surface. A number of workers have obtained evidence of such a surface localization of enzymes. Wilkes and Palmer (1933) and Nelson and Wilkes (1933) have shown that the sucrose 'inverting' enzyme of yeast cells immediately responds in activity to external pH and sucrose concentration as if it were in cell-free solution, thereby strongly suggesting its localization in the cell surface and in direct contact with the external solution. Myrback and Vasseur (1943) have similarly presented evidence for the localization of a lactase on the surface of various yeasts. The surface localization of various phosphatases in animal cells and yeasts has been demonstrated by cytochemical techniques (Dempsey and Deane, 1946; Emmell, 1946) and by the use of isotopic phosphorus (Rothstein and Meier, 1948). Sacks and Altshuler (1942) and Sacks (1944, 1948) have obtained evidence that phosphate absorption by muscle, heart, and liver involves the formation and later degradation on the cell surface of a hexosemonophosphate. This data, together with that cited in our previous paper (Dormer and Street, 1949), all supports the view that certain enzymes, and particularly those concerned with the phosphorylation and dephosphorylation of sugars, are present in the protoplast surface.

The absorption of sucrose by storage tissues (Gawadi, 1935; Saïd, 1941), leaves (Saïd, 1948) and excised roots (Burström, 1941 *b*; Dormer and Street, 1949) is associated with the appearance of a mixture of dextrose and laevulose in the external solution. The laevulose accumulates in a quantity corresponding closely to the theoretical yield to be expected from the sucrose degraded. Sugar absorption involves almost exclusively the dextrose moiety of the sucrose molecule.

Burström (1941 *b*), in his study of the carbohydrate nutrition of excised wheat roots, has advanced the hypothesis, based on Lundegårdh's (1940) studies of the properties of the plasm surface, that sucrose suffers an acid hydrolysis at the acid pH of the root surface, and that sucrose hydrolysis is not directly related to its absorption but is merely a function of the root surface area. Sucrose is, however, toxic to wheat roots and in their presence suffers a very rapid hydrolysis during which it is postulated injurious products arise from the fructose unit. Burström, from a preliminary study of sucrose utilization by Petkuser-rye roots, has suggested that a similar acid hydrolysis occurs, although at only one-tenth the rate observed with wheat, presumably due to the less acid pH at the root surface. The data in this case does not, however, exclude the possibility that a breakdown related to absorption is proceeding simultaneously with an acid hydrolysis. Rye roots apparently grow well when supplied either with dextrose or sucrose. Growth with sucrose is not associated with any toxic symptoms and is more rapid than with an equimolecular dextrose solution. This Burström explains as due to the higher amount of disposable nutrients, although no comparison of growth with equal percentages of the two sugars is recorded.

Gawadi (1935) put forward the view that the appearance of fructose and dextrose in the external solution observed when discs of carrot root tissue were supplied with sucrose was due to the activity of invertase centres on the outer surface of the cytoplasm. Saïd (1941), using discs of carrot and radish root tissue, accepted this hypothesis and in support cited the enhanced sucrose inversion occurring when the thickness of the tissue discs was decreased. These workers visualize invertase hydrolysis to precede utilization of sucrose so that entry of sugar takes place mainly as dextrose and to a smaller extent as fructose. On the basis of this hypothesis Saïd (1948), from his observations on sucrose utilization by detached leaves, has postulated a phosphate activation of the invertase centres.

Whilst certain tissues may effect a hydrolysis of sucrose either by virtue of the acid pH or invertase activity of the plasm surface it is clear, from the following considerations, that a quite different mechanism is involved in sucrose utilization by the excised tomato root:

(*a*) Sucrose supports a growth outstandingly superior to that observed with any other sugar tested.

(*b*) Neither dextrose nor laevulose nor an equimolecular mixture of these two sugars are capable of supporting a growth in any way comparable to that obtained by using sucrose.

(*c*) Sucrose breakdown is quantitatively related to its utilization, one 'dextrose' unit being absorbed for each two molecules of sucrose degraded (Dormer and Street, 1949).

(*d*) The growth responses to changes in the pH of the medium are quite different from those required by the acid hydrolysis hypothesis (Burström, 1941 *b*) but suggest rather the existence of an enzyme controlled mechanism.

Hartt (1944) and more recently Calvin and Benson (1949) have obtained evidence that sucrose synthesis in the green plant takes place by a dephosphorylative condensation. Further, the sugar requirements of *Pseudomonas saccharophila* (Doudoroff, 1940), from which Doudoroff (1943) has isolated a sucrose phosphorylase, are almost identical with those of excised tomato roots. Other studies, previously reviewed (Dormer and Street, 1949), emphasize the possibility that active sugar absorption by animal cells involves phosphorylation at the cell surface followed by dephosphorylation, catalysed by surface-localized phosphatases, as the sugar is liberated into the cytoplasm. It was therefore suggested that the specificity of sucrose as a carbon source for the growth of excised tomato roots and the relationship between sucrose breakdown and sugar absorption would be explained if the mechanism of utilization involved a phosphorolysis of the sucrose at the surface of the absorbing cells. This hypothesis is now supported by the following additional evidence:

(*a*) The similarity between the symptoms of phosphorus- and carbohydrate-deficiency.
(*b*) The demonstration of a phosphate activation of sucrose absorption by phosphorus-deficient roots.
(*c*) The reversibility and proportionality to concentration of the inhibition of excised root growth resulting from phloridzin treatment and the demonstration that this inhibition can be partially counteracted by increasing the sucrose concentration. The view advanced that phloridzin acts as an inhibitor of a phosphorylation involved in sucrose absorption is strengthened by the insensitivity of the attached seedling root to phloridzin treatment.
(*d*) The evidence that dextrose is a competitive inhibitor of sucrose utilization.

Further evidence for the operation of the absorption mechanism here postulated would result from the isolation from excised tomato roots of a sucrose phosphorylase or from the demonstration that these roots are capable of rapidly absorbing and dephosphorylating a hexose phosphate. Investigations along these lines are now proceeding.

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SUMMARY

1. Excised tomato roots are sensitive to the initial pH of the culture medium. Good growth is only obtained with initial pH values within the range 4·2 to 5·0 and there is a clearly defined optimum pH at 4·8 to 4·9.

2. The growth of excised tomato roots causes an alkaline drift in the pH of the culture medium and this factor contributes to the decrease in growth rate which occurs with time.

3. Excised tomato roots have a low requirement for inorganic phosphate. The symptoms of phosphorus-deficiency are similar to those associated with carbohydrate deficiency. A rapid restoration of the sucrose absorbing ability of phosphorus-deficient roots results from an external supply of phosphate.

4. Phloridzin inhibits the growth of excised tomato roots. No similar inhibition is observed using the attached seedling root. The phloridzin inhibition of excised root growth is reversible and proportional to the concentration of phloridzin used and is partially counteracted by increasing the sucrose concentration.

5. Excised tomato roots readily utilize sucrose as a carbon source. Dextrose is only able to support a very slow rate of growth and yields roots showing symptoms of carbohydrate deficiency. Galactose, mannose, arabinose, xylose, rhamnose, laevulose, maltose, and raffinose cannot be utilized as carbon sources.

6. Laevulose, galactose, xylose, and maltose are markedly toxic to excised roots simultaneously supplied with sucrose. Dextrose appears to act as a competitive inhibitor of sucrose utilization.

7. Two per cent. sucrose was superior to the other concentrations tested. Symptoms of carbohydrate deficiency were apparent when the concentration of sucrose was reduced to 0·5 per cent. A concentration of 4 per cent. sucrose or higher caused a marked check in growth rate. Using raffinose, which exerts no deleterious effect on the growth of roots simultaneously supplied with sucrose, it was possible to show that the growth obtained with 1 per cent. sucrose can be enhanced by raising the osmotic pressure of the medium and that the unfavourable effect of media of high sucrose content is not a consequence of their higher osmotic pressures.

8. A discussion is presented of the bearing of the above data on the hypothesis that the mechanism of sucrose utilization by excised tomato roots involves its phosphorolysis at the surface of the absorbing cells.

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Inequality in Size of the Male Nuclei in the Genus *Ephedra*

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With Plates IX and X and one Figure in the Text

INTRODUCTION

IT has been previously stated that the two male nuclei in *E. saxatilis* Royle are unequal, the one in front in the pollen tube being smaller, followed in the rear by the larger one (Mehra, 1938). In the same paper the male nuclei in *E. foliata* Boiss. were described as equal. In a later communication dealing with the karyotypes in some species of the genus, *E. altissima* Desf. and *E. intermedia* Shrenk. et Mey. were again described as possessing unequal male nuclei, the male nucleus towards the stalk nucleus end of the grain being invariably bigger than the other in both the species (Mehra, 1947). The inequality in size of male nuclei is also known in the other genera of the Gnetales. Thus Pearson (1929) found unequal male nuclei in *Welwitschia* and the same is known to be the case in some *Gnetum* species. Unequal male nuclei are not confined only to Gnetales. In Coniferales Ferguson (1904) reported unequal male nuclei in *Pinus strobus*. While unequal male nuclei are thus recorded from a number of gymnosperms it is not known what determines this inequality in size. Chamberlain (1935, p. 319) stated: 'In Gymnosperms, in many cases the two sperms differ in size but whether they differ in chromatin content or in any other way, has not been determined either in Gymnosperms or in any other group.' The present study was undertaken to inquire into this problem.

E. foliata Boiss., *E. altissima* Desf., *E. intermedia* Shrenk. et Mey., *E. saxatilis* Royle, *E. sinica* Stapf, and *E. likiagensis* Florin form the subject of the present investigation.

The pollen grains in each case were germinated artificially on glass slides in the natural mucilage secretion that oozes out of micropylar canals of ripe receptive ovules in a manner already described (Mehra, 1947). The fixatives employed were Flemming's weak and Allen's modification P.F.B.$_{15}$ of Bouin's fluid and staining was done in Haidenhain's iron-alum haematoxylin. Besides the facts dealing with mitosis of the body-nucleus leading up to the formation of male nuclei, a study of the cytoplasmic inclusions in the male gametophytes has also been undertaken. The fixatives employed in this latter study are mentioned at the proper places in the text.

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OBSERVATIONS

It is well known that the ripe pollen grain in *Ephedra* consists of two evanescent vegetative cells towards one pole, a large tube nucleus towards the opposite end, and a circular body-cell in the centre on whose periphery is embedded the stalk-nucleus. The position of the stalk-nucleus as a rule is along the long axis of the grain towards the vegetative end, but this is not consistently the case. In many cases the stalk-nucleus may lie on the oblique axis or even along the transverse axis at the periphery of the body-cell. The body-cell cytoplasm is very dense compared to that of the rest of the grain outside it and is bounded externally by a thin membrane. There are invariably embedded in it a large number of small circular bodies, designated as 'spherules' in the body of this paper, which glisten in the living pollen grains in reflected light. The cytoplasm of the gametophyte outside the body-cell is vacuolated and the characteristic 'spherules' are absent.

The body-nucleus is somewhat larger than the stalk-nucleus and like it is highly compact and dense. The entire space is occupied by chromosomes occurring in the form of a knot with very little karyoplasm. Even in the best fixed preparations it is impossible to make out the internal structure of the individual chromosomes, but it can be stated that they are condensed as in an interkinetic nucleus.

As soon as the grains are put on the mucilage secretion they absorb the medium, swell in size, and the prophasic changes start in the body-nucleus. It grows considerably in size and the individual chromosomes elongate. In the mid-prophase their primary constrictions are visible and also their dual nature. It is observed that they are polarized, with the primary constrictions of all facing the end opposite the stalk-nucleus, which means that the chromosomes have undergone no change in their orientation after the previous division. At late prophase the two chromonemata of every chromosome are observed to be interconnected by a few pale staining processes traversing the intra-chromonematal matrix.

About this time the exine ruptures by two longitudinal fissures starting from the tube-nucleus end of the grain and the spindle-shaped gametophyte bounded by the intine jerks out into the germinating medium while the exine undergoes torsion.

On the dissolution of the nuclear wall at the end of the prophase the chromosomes emerge in the form of a polarized bouquet with the free ends of the chromosomes pointing towards the stalk-nucleus (Pl. I, Figs. 1, 2). Evidently the chromosomes have retained the same disposition as at the end of the previous telophase during the period of interkinesis. The chromosomal knot now gradually loosens as the arms spread out and at the same time spindle-fibres are organized towards the two ends, making the position of the kinetochore regions of the chromosomes as the equator. The portion of the spindle towards the tube-nucleus end is the first to be observed (Pl. I, Fig. 5) and is organized from the cytoplasm of the body-cell exclusively, while the spindle-half towards the opposite end is late in appearance and seems to be formed in part at least from the karyoplasm.

The spindle-axis thus incidentally points towards the stalk-nucleus. If the latter lies on the longitudinal axis of the grain, the spindle also assumes the same position, while if it lies on the oblique axis or transversely the spindle-axis follows likewise (Pl. IX, Figs. 3, 6, 8, 9). Apparently, therefore, it appears as if the stalk-nucleus governs the orientation of the spindle-axis of the dividing body-nucleus, but in reality it is because the chromosomes organizing the body-nucleus have not undergone a different orientation from their previous telophasic position, have reappeared in the same fashion, and the spindle is organized in such a way that the position of their primary constrictions becomes its equatorial region.

In *E. foliata* and *E. sinica* the spindle is homopolar, i.e. the two ends of the poles are similarly converging. On the other hand, in *E. altissima*, *E. intermedia*, and *E. saxatilis* the spindle is heteropolar with stalk-nucleus end flat and truncate while the opposite end converges to a point. It is this heteropolar nature of the spindle that determines the inequality in size of the male gametes in these species, as will be soon clear.

At the equatorial plate the daughter chromosomes split and move towards either pole led by their kinetic bodies. In some preparations a centromere granule at the region of primary constriction in the chromosomes at this stage is very clearly observed.

In one case in *E. sinica* a fragment is seen cast off the spindle region while the chromosomes are at the equatorial plate (Pl. X, Fig. 11). How this has come to be it is not possible to say.

The internal structure of the daughter chromosomes at mid-anaphase is not very clear, but there are indications of the presence of two chromonemata, particularly near the free arms. At the late anaphase the spindle elongates, simultaneously undergoing a lateral contraction so that the daughter-chromosome groups are pulled apart. Not infrequently the two arms of a long chromosome may still be joined, forming an equatorial bridge (Pl. IX, Fig. 8 upper, and Pl. X, Fig. 13). The spiral nature of the chromosomes is very obvious in certain preparations and it is observed that the chromonema is certainly not continuous.

At telophase the individual chromosomes undergo further contraction by spiralization. Where the separation of a chromosome arm is delayed, the latter is still observed projecting out from the otherwise smooth contour of the chromosomal clump (Pl. X, Fig. 12).

Formation of the male nuclei

In species with heteropolar spindle the condition of the chromosomes when they arrive at the two poles is different. At the flat truncate pole (facing the stalk-nucleus) the chromosomes, for obvious reasons, remain rather scattered with their free arms spreading (Pl. IX, Fig. 9 left, and Pl. X, Fig. 13). The opposite pole being converging, the chromosomes almost reach the same point and are thus relatively more compacted.

When the nuclear wall is organized around the two groups of chromosomes, obviously the size of the nucleus at the flat end of the spindle where rather scattered chromosomes are being enclosed is greater than the one on the opposite end (Pl. IX, Figs. 6, 7, and Pl. X, Fig. 10). Thus there is no difference whatsoever in the chromatin content of the two nuclei, the inequality being determined by the more dispersed nature of the chromosomes at one end than at the other, which in turn is determined by the heteropolar nature of the spindle. But the amount of karyolymph in the larger nucleus is greater than in the smaller.

In *E. foliata* and *E. sinica* where the spindle is homopolar the two male nuclei formed are equal in size. This is anticipated even at telophase, where the chromosomal knots at two poles are observed to be of the same size (Pl. X, Fig. 4, and Pl. IX, Fig. 12).

During the formation of the male nuclei the chromosomes loose their staining capacity gradually and in proportion to this the light-grey substratum in which they are embedded assumes a pale appearance. It seems as if some substance is secreted out of the chromosomes which on reacting with the surrounding medium changes its staining reaction from greyish to pale. The pale substance is the karyolymph and around it is deposited the nuclear wall.

The male nuclei then gradually increase in size. In species where they are unequal the same proportional difference in size is maintained during their growth.

During the growth of the male nuclei the chromosomes, which had previously undergone a high degree of contraction due to spiralization at telophase, also lengthen out. Although they stain feebly, yet their outlines are clearly visible. The chromonema threads of these is not continuous, but there are gaps at intervals.

After the organization of the male nuclei the spindle disappears, starting from the polar ends (Pl. IX, Figs. 6, 7).

E. altissima is a tetraploid and produces besides the normal $2n$ pollen a small percentage of $4n$ pollen (Mehra, 1947). The two male nuclei in the normal pollen are unequal. Pl. X, Fig. 10, shows a giant $4n$ pollen in the same species, and the male nucleus facing the stalk-nucleus is observed to be bigger than the other as in the normal grain. Similar size differences in the two nuclei are maintained in the tetraploid pollen of *E. saxatilis* and *E. intermedia*, which are characterized by the inequality in size of the male nuclei in their normal $2n$ pollen.

In *E. likiagensis* the male nuclei may be equal or somewhat unequal with none of the exaggerated difference in size observed in *E. altissima*, *E. intermedia*, and in particular *E. saxatilis*.

At the end of the formation of the male nuclei the pollen tubes are put out, the details of which have already been described (Mehra, 1938).

In artificial culture and also in the pollen tube developed normally in the pollen chamber in the ovule in *E. saxatilis* and *E. intermedia* it is observed that the smaller gamete always precedes the larger one (unpublished data), and this is presumably the one involved in fertilization.

DURATION OF THE MITOTIC CYCLE

The duration of the mitotic cycle varies with the nature of the medium in which the germination of the pollen occurs and the temperature. In natural mucilage secretion the time taken is much less than in any concentration of saccharose solution (Mehra, 1938).

It takes approximately 5¼ hours for complete mitosis to be effected in natural mucilage at a temperature of 92–3° F. in *E. foliata*, while this is reduced to 3 hours only at 96–8° F.

The duration of each mitotic stage was determined for *E. foliata* pollen germinated on natural mucilage secretion at 92–3° F. The culture was started at 11 a.m. and the stages in division were directly observed under the microscope at short intervals. These were further confirmed by staining a test sample with acetic-iodine green.

The prophase lasted for 2 hours (till 1 p.m.). The spindle was organized and the chromosomes assumed their position in the form of an equatorial plate by 2.37 p.m., thus taking 1 hour and 37 minutes. The movement of the daughter chromosomes to the two poles was effected in only 1 hour and 13 minutes (by 3.50 p.m.). It took another 15 minutes for the male nuclei to be organized. The total period of mitosis thus lasted for a little over 5 hours at 92–3° F.

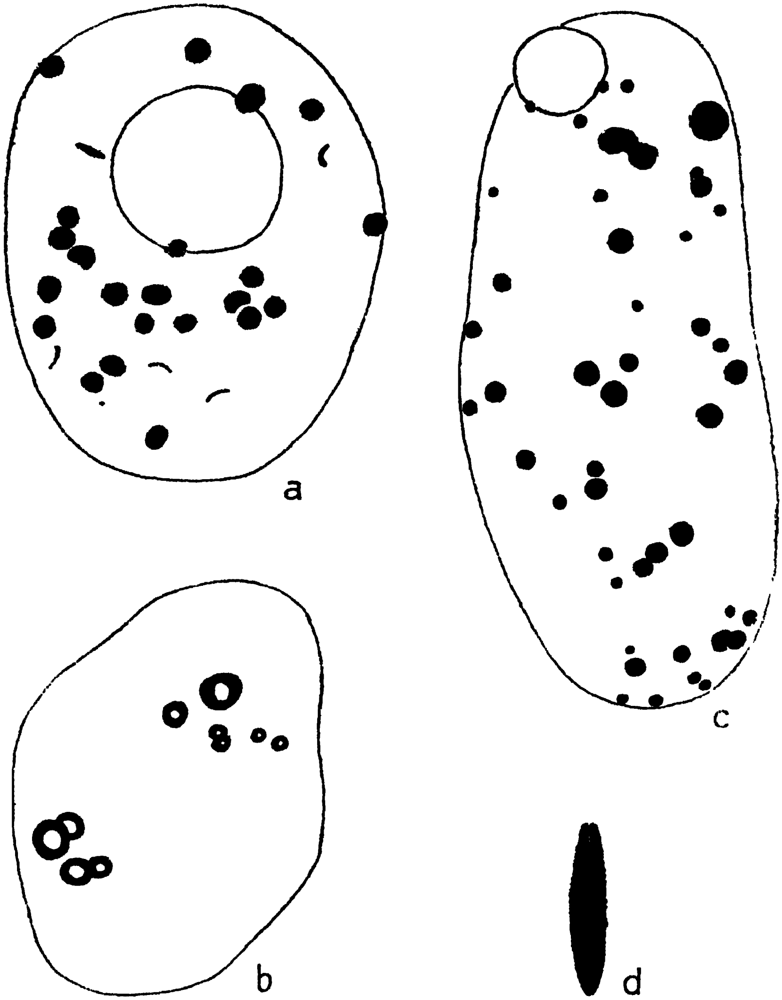
CYTOPLASMIC INCLUSIONS

It has already been stated that the cytoplasm of the body-cell is very dense and is devoid of any vacuoles. In it are present two types of bodies:

1. Small spheres or 'spherules' which occur invariably.
2. Spindle-shaped bodies or 'spindlets' which are of sporadic occurrence.

Spherules

These are of invariable occurrence in the body-cell cytoplasm of all the six species of the genus under investigation and their presence must therefore be regarded as a rule in the genus (Pl. X, Figs. 13–16, and Text-fig. 1 *a* and *c*). Outside the body-cell cytoplasm they are absent. Because of their high refractive index compared to the cytoplasm in which they are embedded, they appear as highly glistening bodies in reflected light under high-power or oil-immersion lens in the living pollen grains, particularly when the exine is cast off during their germination. In size the spherules vary from mere dots to those of considerable size. Their number in a body-cell varies in accordance with their size. When dot-like they are quite numerous, but when of bigger size they are comparatively fewer. In a body-cell all the spherules may be dot-like or there may be a slight variation in size or the majority may be of uniformly large size with a few dot-like intermixed, or in still other cases there may be a good deal of variation in size (Text-fig. 1 *c*). The larger of these spherules



TEXT-FIG. 1. *a. E. foliata*, body-cell showing the entire spherule and spindlet material. The body-nucleus is shown in outline. From living material × 1,840. *b. E. foliata*, body-cell from a living gametophyte after it had been mounted in water for 30 minutes. Spherules have considerably swollen and their vacuolous nature intensified × 1,840. *c. E. altissima*, body-cell fixed in Flemming's W.R.T. for *Gasteria* and stained in iron-alum haematoxylin. Stalk-nucleus shown in outline and the body-nucleus which is at metaphase not drawn. Entire spherule material shown × 1,840. *d.* Spindlet from preparation shown in fig. 17 of Pl. II. Highly magnified.

are particularly refractile in the living condition. They may be irregularly scattered singly or in several irregular groups. Often they form beautiful chains (Pl. X, Figs. 14, 15), but all these arrangements are merely incidental. During the division of the body-nucleus they are never present in the spindle zone, and when the two male nuclei are organized the spherules may be aggregated round them, but this is of no special significance.

The spherules are particularly susceptible to fixatives containing acetic acid and are therefore lipoidal in nature. In Bouin's fixative they never appear (Pl. IX, Figs. 1–4, 8, 9). Beautiful preparations are, however, obtained after fixation in Flemming's for *Gasteria* with only traces of acetic acid followed by staining in iron-alum haematoxylin (Pl. X, Figs. 13–15), or when Flemming's weak fixative with traces of acetic acid is used with subsequent staining in iron-alum haematoxylin.

The spherules are undoubtedly vesicular in nature. This is observed in the larger spherules in the living condition when the outer rim appears dense and more refractive than the interior. In fixed preparations stained with iron-alum haematoxylin and properly differentiated the same thing is observed. If the living grains are immersed for a period of half an hour in ordinary water, the cytoplasm of the gametophytes gradually undergoes a process of senescence, but during this period some of the larger spherules swell considerably in size and their vesicular nature becomes quite conspicuous (Text-fig. 1 *b*).

In fixed preparations often two or more spherules are observed fused together into an irregular mass (Text-fig. 1 *c*). Whether or not this has something to do with the action of fixative on closely situated spherules is not clear. This seems possible in view of the fact that under the action of the fixative the spherules often loose their perfectly spherical outline and become hemispherical or lunar or develop angularities.

The total spherule material in the body-cell of *E. foliata* is about half of that in the other five species. This is in accordance with the size of the pollen grains in the two cases, which in turn is governed by the fact that the pollen grains in *E. foliata* possess a set of seven chromosomes while those in *E. intermedia*, *E. sinica*, *E. likiagensis*, *E. altissima*, and *E. saxatilis* each possess a set of fourteen chromosomes (Mehra, 1947).

Again it has been found that in *E. intermedia*, *E. altissima*, and *E. saxatilis* besides the normal grains with fourteen chromosomes a small percentage of giant grains with twenty-eight chromosomes is also met with (Mehra, loc. cit.). The spherule material in such giant grains is again roughly double to that met with in the normal grains of these species.

It has been stated that spherules are lipoidal in nature. Fixatives containing acetic acid do not preserve them. If a drop of dilute acetic acid is run under the coverslip containing fresh, living, germinating pollen grains underneath, the glistening spherules immediately disappear. They are Feulgen-negative and give negative tests for starch, proteins, oils, and fats.

It is difficult to state the exact nature of spherules. They correspond most closely to the microsomes or lipide granules of Guilliermond (1941), but there is a distinctive difference in that the spherules are vacuolar whereas the microsomes are not. They are not the mitochondria because of their much larger size, their highly refractive nature, and their inability to take the intravitem stain Janus green, although they resemble the mitochondria in being vacuolar as the latter sometimes are (Guilliermond, loc. cit.). Also with fresh coverslip osmication in 2 per cent. solution of osmic acid for 20 hours they take only a very feeble stain, whereas the mitochondria stain jet black. They most certainly do not belong to the category of either plastids or vacuome.

Spindlets

These do not seem to be of constant occurrence in the body-cell cytoplasm. In the material fixed in Flemming's W.R.T. for *Gasteria* and stained in iron-alum haematoxylin they may be observed in some grains while they are absent in others.

When young these bodies are in the form of straight or slightly curved threads (Pl. II, Fig. 16, and Text-fig. 1 *a*), but they grow in size and when mature appear in the form of short spindlets. Often the two spindlets may be observed in such close approximation with each other along their longitudinal axis that it gives the impression that they multiply by longitudinal fission. In Text-fig. 1 *d*, drawn from the preparation shown in Pl. X, Fig. 17, a fairly mature spindlet is observed which appears to be in an early stage of fission, as indicated by the non-staining line in the middle at one of the poles. This is not so clear in the photograph.

The number of spindlets when present are variable. There may be just one large spindlet in the entire body-cell or there may be three, four, or more relatively smaller ones (Pl. X, Figs. 17, 16 respectively). The distribution of these spindlets, like the spherules, is irregular.

The nature of these spindlets or their function is not known. Whether they are of the nature of crystals is problematical.

The cytoplasm outside the body-cell is thin and highly vacuolated. There are no spherules or spindlets present. With very dilute intravitem neutral red solution the vacuoles take reddish coloration and soon their colloids are precipitated in the form of small granules which perform rapid Brownian movements.

SUMMARY

The development of male nuclei from the division of the body-cell nucleus has been studied in six species of the genus *Ephedra*.

In *E. foliata* and *E. sinica* the two male nuclei are of the same size, in *E. likiagensis* the male nuclei may be equal or unequal, but in the latter case there is no marked difference, while in *E. altissima*, *E. intermedia*, and *E. saxatilis* they are markedly unequal, particularly in the last species.

The inequality, where present, is due to the heteropolar nature of spindle of the dividing body-nucleus. The spindle-half towards the stalk-nucleus end is late in appearance, truncate and blunt at the pole, in contrast to the spindle-half on the other side which is converging. The chromosomes when they reach the pole at the blunt end are consequently spread compared to those on the opposite side where they form a relatively compact mass at telophase. The nucleus formed towards the stalk-nucleus end, therefore, is invariably bigger than its sister on the other end. There is thus no difference in the amount of

chromatin in the two nuclei, but there is obviously some in the amount of karyolymph. The two male nuclei, if unequal, maintain this difference even during their further growth.

Species which possess unequal male nuclei in the *x* pollen grains exhibit the same difference even in the $2x$ ones if present.

There are invariably present in the dense cytoplasm of the body-cell spherical bodies vasicular in nature which glisten in reflected light in fresh condition. Their chemical nature is lipoidal.

In addition there occurs sporadically in the body-cell cytoplasm another type of body here called 'spindlets'. When young they are in the form of straight or slightly curved threads of short lengths which on maturity assume spindle-shaped outline.

The cytoplasm outside the body-cell is vacuolated and does not contain any of the two types of bodies stated above.

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EXPLANATION OF PLATES

Illustrating P. N. Mehra's article 'Inequality in Size of the Male Nuclei in the Genus *Ephedra*'. All figures from untouched photographs.

Figs. 1–12 from preparations fixed in Allen's modification P.F.B.$_{15}$ of Bouin's fixative. Figs. 13–17 from preparations fixed in Flemming's W.R.T. for *Gasteria* with only traces of acetic acid. In all cases the exine has been cast off.

PLATE IX

Figs. 1–4. *E. foliata.*

Figs. 5–7. *E. intermedia.* Mark in Fig. 5 the converging spindle-half on the side opposite the stalk-nucleus, while the one towards the stalk-nucleus end is not yet organized. In Figs. 6 and 7 unequal male nuclei are observed.

Figs. 8–9. *E. saxatilis.*

PLATE X

Fig. 10. *E. altissima.* A $4n$ pollen grain showing unequal male nuclei.

Figs. 11–12. *E. sinica.* Mark the equal male nuclei in Fig. 12.

Figs. 13–14. *E. saxatilis*, showing the spherules.

Fig. 15. *E. intermedia.* Only spherules are present and no spindlets.

Fig. 16. *E. intermedia*, showing both spherules and spindlets.

Fig. 17. *E. intermedia.* A big spindlet is observed with a few spherules, the remaining spherules being out of focus.

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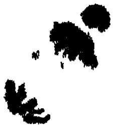
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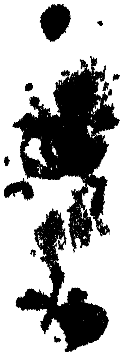
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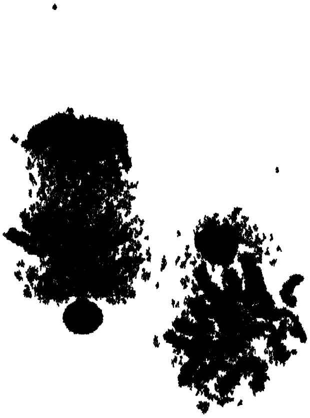

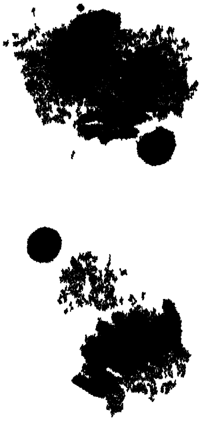
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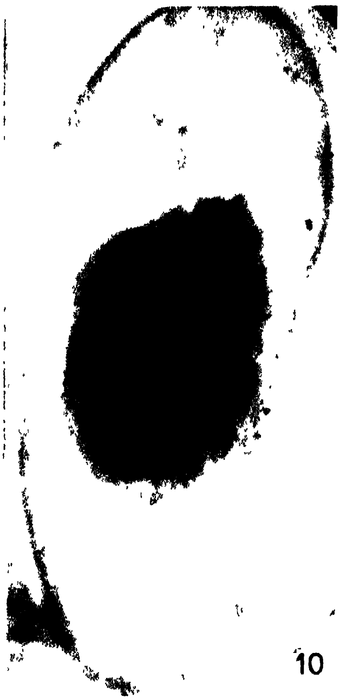

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P. N. MEHRA

Alternation of Generations and Heteroecism in *Puccinia versicolor*

BY

M. J. THIRUMALACHAR

AND

M. J. NARASIMHAN

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With Plate XI and 1 Figure in the Text

INTRODUCTION

HETEROPOGON *contortus* (L.) Beauv., commonly known as spear-grass, is widely distributed through the tropics and subtropics. It is a common grass of the dry pasture-lands in south India. *Puccinia versicolor* Diet. and Holway is also widely distributed on this grass in India. A detailed field and cultural study has revealed that the rust is heteroecious with pycnial and aecial stages on *Canthium parviflorum* Lam. (*Plectronia parviflora* Bedd.), a common shrub of the dry waste lands.

MATERIAL AND METHODS

Material for spore germination and inoculation studies was collected in Bangalore during different seasons and stored in paper bags at room temperature. The aecio-, uredio-, and teliospores were germinated on slides by adopting the technique suggested by Thirumalachar (1940).

For inoculation experiments the plants were grown in pots under rust-free conditions and the usual methods of inoculation were followed. For cytological studies the material was fixed in formalin acetic alcohol or Allen's modification of Bouin's fluid, and microtome sections of 8 to 10μ thickness were cut and stained with Heidenhain's iron-alum haematoxylin or Newton's iodine gentian violet.

OBSERVATIONS

Life-cycle on Heteropogon contortus

Uredia

Morphological details of the uredia and telia are already given by Dietel and Holway (1897), the Sydows (1904), and Doidge (1926). The uredia first appear on the spear-grass in July–August, developing on reddish-yellow spots. The sori are yellowish-white, erumpent and pulverulent. The uredio-spores are ovate-globose, hyaline, and with an epispore which is echinulate and irregularly thickened to impart a stellate appearance in the interior

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(Text-figs. 1 and 2). The germ-pores are indistinct and become apparent only during germination.

The urediospores readily germinate in water, developing long germ-tubes (Text-fig. 3). Spores from freshly erupted sori when placed for germination on slides at room temperature (about 24° C.) gave between 90 and 100 per cent. germination. Viability of the urediospores in material stored under laboratory conditions showed that it decreased gradually and no viable spores were observed after 30 days.

The urediospores incite secondary infection of the spear-grass and there is a rapid spread of the rust in the field from August to November. After the development of the telia, secondary uredia are no longer produced. The host plants begin to dry up by the end of April, and in May and the early part of June no viable urediospore material can be collected in the field. The question of over-summering of the rust in the uredial stage is of secondary importance, since the uredial stage is easily initiated by aeciospore infections from the alternate host.

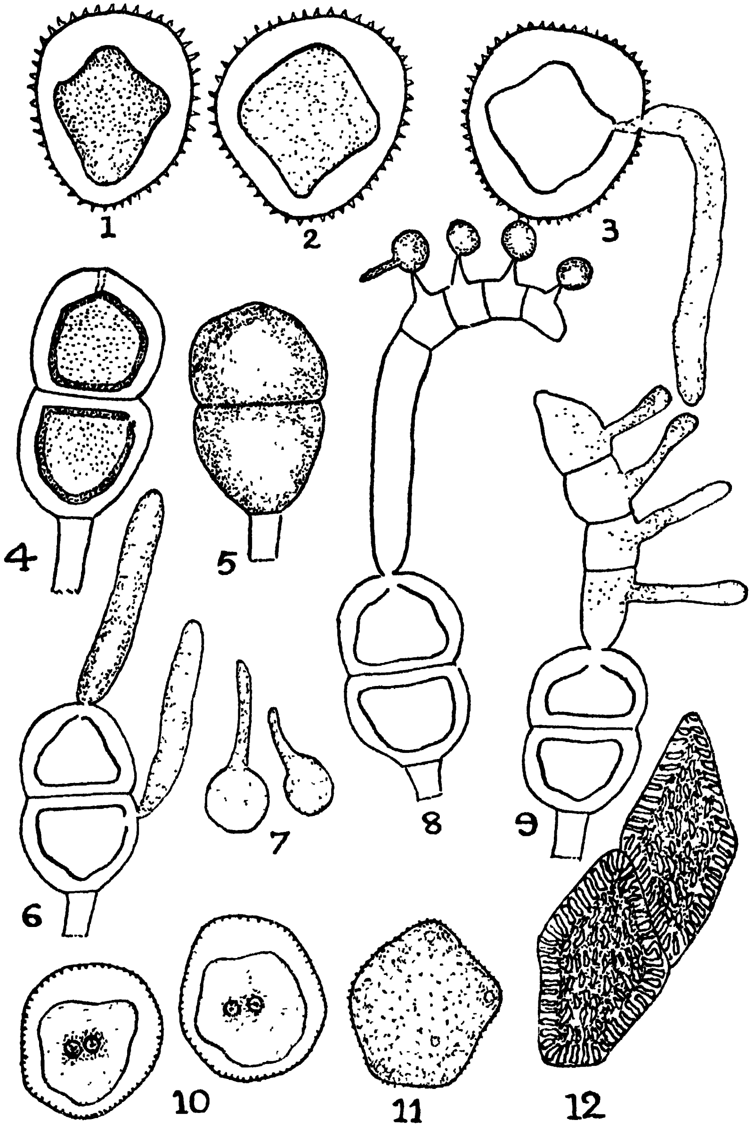
Inoculation experiments were carried out using urediospores. Spear-grass taken from clumps growing in rust-free areas or from seedlings were inoculated. The development of secondary uredia were noticed after 15 to 20 days. Doidge (1926) reports *Themeda triandra* Forsk., *Hyparrhenia* sp., and *Monocymbium ceresiaeforme* Stapf as additional grass hosts for *Puccinia versicolor*. No cross inoculations using urediospores were carried out in the present study to check the host range.

Telia

Telia follow uredia in development in December, appearing as tiny black specks. In April and May, when the production of secondary uredia has ceased, the telia can be collected in abundance. In early stages the teliospores are developed within the uredia and later completely replace them. Mature teliospores are reddish-brown, oblong-ellipsoid, slightly or not constricted at the septa (Text-figs. 4 and 5), and smooth. The walls are up to 2·5 μ thick and possess a single germ-spore in each cell. Cytological studies indicate that the young spores are binucleate, the two nuclei fusing in the mature spore.

The teliospores have been germinated in large numbers. Spores from freshly collected material in December and January yielded very poor results. Not more than 5 per cent. of the spores were seen to germinate after 5 to 6 days in moist chambers. The teliospore material collected in March was divided into two lots, one spread in shade in the field on April 1, 1949, and thus exposed to natural environmental conditions, the other lot stored in the laboratory at room temperature. The temperature in shade between April 1 and May 15, 1949, in Bangalore showed an average minimum of about 70–3° F. and an average maximum of about 93–5° F.

Germination tests were carried out at various intervals during this period. The percentage of teliospore germination was very low during April and gradually increased towards the middle of May. In tests carried out on May 15



Text-figs. 1–12. Figs. 1 and 2. Urediospores (×800). Fig. 3. Germinating urediospores (×800). Figs. 4 and 5. Teliospores (×750). Fig. 6. Early stage in teliospore germination (×750). Fig. 7. Germinating sporidia (×750). Fig. 8. Germinating teliospore bearing sporidia (×750). Fig. 9. Abnormal teliospore germination (×750). Figs. 10 and 11. Aeciospores (×800). Fig. 12. Peridial cells (×1,000).

the teliospores from material exposed to natural environmental conditions germinated 80 to 100 per cent., the sporidia being formed within 10 hours. In contrast, the teliospores in material stored in the laboratory during the same period showed very poor germination, not amounting to more than 4 per cent.

Teliospores from material after-ripened in the field germinated readily when placed in moist chambers. Both cells of the teliospore germinate (Text-fig. 6), producing a four-celled promycelium (Text-fig. 8). The sporidia are globular, hyaline, and germinate *in situ*, developing long germ-tubes (Fig. 7) or occasionally secondary sporidia. In stained preparations the sporidia show a single nucleus. Abnormal teliospore germinations due to excess of moisture have been noticed in several instances. The sterigmata in such cases elongate without sporidial formation (Text-fig. 9).

Life-cycle on Canthium parviflorum

The existence of an alternate host bearing the pycnial and aecial stages became evident after intensive field studies for several seasons. In July the uredial stage usually appeared first on clumps of spear-grass near *Canthium parviflorum*, a thorny shrub belonging to the Rubiaceae. An unconnected *Aecidium* closely agreeing with *Aecidium plectroniae* Cooke has been collected by the writers for several years. The appearance of the aecial stage on *Canthium* in the lawns following the monsoon rains in July was a strong indication that it was possibly connected with some grass rust.

In order to confirm this suspected heteroecious cycle of *Puccinia versicolor*, after-ripened teliospores were germinated on slides and used for inoculating young unfolding leaves of *Canthium parviflorum* grown in the laboratory under rust-free conditions. The inoculated plants were enclosed in moist chambers for 48 hours, after which they were placed outside for further observation. As many as sixty-five separate inoculations were carried out and all yielded successful infection. Several controls all remained healthy.

The first symptom following successful infection became evident after 8 days by the development of swollen pale greenish patches on the young leaves. The infection causes marked hypertrophy. Pycnia, which are amphigenous, appear first as greenish viscid specks. Sections through the leaf indicate that they are subepidermal, angular, deep seated, with few ostiolar paraphyses (Pl. XI, A). The aecia follow the pycnia in development after a period of 12 to 18 days (Pl. XI, C and D). They are mostly hypophyllous, erumpent, and pulverulent, white, with well-developed peridia (Pl. XI, B). The cells of the peridia are firm, thick-walled, polygonal to rhomboidal with folded rugose thickenings (Text-fig. 12). The aeciospores are white in mass, developed in chains, angular to polygonal, slightly thickened at the apex, and possess hyaline contents (Text-fig. 10). The epispore is pitted, presenting a verruculose appearance and possessing 6 to 7 scattered indistinct germ-pores (Text-fig. 11). In stained preparations the young pycnia and aecia are evidently haploid. The aeciospores are binucleate, but the initiation of the dicaryophase was not observed.

Aeciospores from freshly erupted sori germinate with long germ-tubes within 24 hours when placed in moist chambers. Such spores were used for inoculating spear-grass plants, which were enclosed in moist chambers for 48 hours and then transferred to a greenhouse for further observation. Evidence of successful infection became manifest after 10 days by the appearance of reddish-yellow specks on the blades of spear-grass. The development of uredia took place 15 to 20 days after inoculation and thus completed the cycle of development.

DISCUSSION AND SUMMARY

The heteroecious nature of *Puccinia versicolor* was first inferred from field studies and later confirmed by cross-inoculation experiments. The aecial stage on *Canthium parviflorum* was produced in the laboratory nearly 2 months in advance of its natural appearance in the field.

As regards the identity of the aecial stage, no less than nine unconnected *Aecidium* states have been described on the host genus *Canthium.* The aecial stage of *Puccinia versicolor* comes nearest to *Aecidium plectroniae* Cooke. No aecial stage has hitherto been recorded on *Canthium* in India.

It is interesting to note that in places where the uredial and telial stages on *Heteropogon contortus* have been reported in India and South Africa, the aecial stage on *Canthium* also occurs in the same locality. Several other *Aecidium* species such as *A. plectoniicola* P. Henn., *A. baumianum* P. Henn., *A. busseanum* P. Henn., *A. baileyanum* Syd., *A. incomparabile* Syd., which are recorded on *Canthium* spp. from different parts of the world, resemble the aecial stage of *Puccinia versicolor* to some extent. Some of these are possibly connected with other rusts on Andropogoneae.

In conclusion the writers wish to express their gratitude to Dr. G. R. Bisby, Commonwealth Mycological Institute, Kew, for suggestions and criticism.

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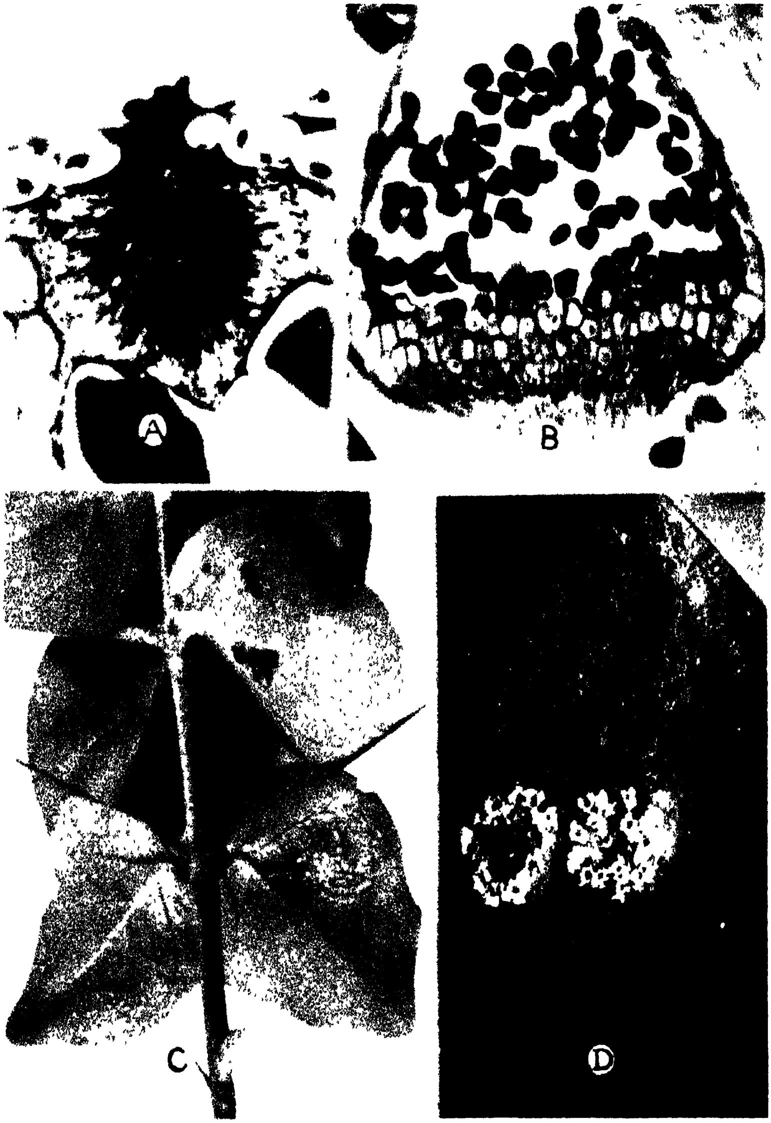
EXPLANATION OF PLATE

Illustrating M. J. Thirumalachar's and M. J. Narasimhan's article 'Alternation of Generations and Heteroecism in *Puccinia versicolor*'

A. Pycnium. × 200.
B. Aecium. × 150.
C. Aecia produced on *Canthium parviflorum* by artificial inoculation. × 1½ nat. size.
D. Enlarged view of the same. × 7.



THIRUMALACHAR and NARASIMHAN

Studies on the Germination of the Seeds of *Striga Hermonthica*

I. The Influence of Moisture-Treatment, Stimulant-Dilution, and After-Ripening on Germination

BY

K. B. VALLANCE

(The Hartley Botanical Laboratories, University of Liverpool)

With ten Figures in the Text

INTRODUCTION

IT has been known for many years that the optimum germination of the seeds of some angiospermous root parasites will only take place in the presence of some stimulant exuded from the roots of suitable host plants. The effects of moisture-treatment and stimulant-dilution on the germination of the seeds of the root parasites *Orobanche speciosa* and *Striga lutea* have been investigated by Chabrolin (1938) and Brown and Edwards (1944, 1946) respectively. These workers showed that if the seeds of these root parasites were exposed to moisture, prior to the application of a suitable stimulating solution, the rate of germination was enhanced, and also that, as moisture treatment was extended, a stage was reached at which the stimulating solution could be diluted 1,000 times without significantly affecting germination.

The present paper relates to an investigation of the effects of moisture-treatment and stimulant-dilution on the germination of the hemi-parasite *Striga hermonthica*. It was thought best, however, to consider these effects in relation to the possibility that the seeds might require after-ripening in dry storage. In this latter respect the present investigation differs from those of earlier workers.

To enable the possible influence of after-ripening to be investigated, seeds harvested in different years were employed. These seeds were harvested in the Sudan in 1940 and 1941 and in Tanganyika in 1945 and 1948. For supplying this material the author wishes to acknowledge his indebtedness to Dr. F. W. Andrews, the Gezira Research Farm, the Anglo-Egyptian Sudan, and to the Empire Cotton Growing Corporation.

EXPERIMENTAL METHODS

The stimulating solution employed in the germination studies consisted of glass-distilled water in which the roots of *Sorghum vulgare* (Sudan grass), a suitable host plant, had been allowed to grow for a constant period. The

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method of preparation was similar to that of Brown and Edwards (1944). Seeds of *Sorghum vulgare* were allowed to germinate for 2 days at 32° C. Fifty of the resultant seedlings, of approximately equal size, were placed on a raft made from one layer of surgical gauze and a ring of egg-shell glass tubing. This raft was floated on 50 ml. of glass-distilled water inside a staining-jar and the jar sealed by means of a rubber stopper. The jars and their contents were then maintained at 22° C. in an incubator for 5 days. The liquid inside the jars was then ready for use as the stimulating solution. Immediately prior to its use the contents of three jars were mixed, for it was considered that by this means possible variations in the concentration of the stimulating substance would be reduced. It was found necessary to use the mixture immediately, for on exposure to the air it rapidly lost its activity.

The method used for investigating the effects of moisture-treatment and stimulant-dilution on the germination of the seeds of *Striga hermonthica* was adapted from that of Brown and Edwards (1944). It involved two distinct yet complementary treatments. Following the terminology of Brown and Edwards (1944) the first treatment was termed 'pretreatment'. To effect pretreatment the seeds were moistened with distilled water and maintained at a constant temperature for varying periods. The temperature of pretreatment was not the same in all experiments, and altogether seeds were pretreated at temperatures of 12° C., 22° C., 27° C., and 32° C.

After periods of pretreatment varying from 1 to 51 days samples of seed were removed from the incubators. Each sample of seeds was divided into two lots and half were treated with the stimulating solution and half were used for the control series. In the control series distilled water was used instead of the stimulating solution. The seeds exposed to the stimulating solution and those of the control series were then maintained at 32° C. for 48 hours. This latter treatment constituted the second phase of the germination method.

The amount of seed used for any experiment in which the seed required pretreatment was standardized at 0·5 g. For pretreatment the 0·5 g. sample was placed in a 2×2 Pyrex sintered glass Gooch crucible which was then put into a 150-ml. Pyrex beaker, and the beaker was filled with glass-distilled water up to the lower level of the sintered disc. After this the beaker and its contents were placed in an incubator maintained at one of the appropriate temperatures mentioned above. Fungal growth inside the crucibles was minimized by washing the seeds with distilled water at the same temperature as the seeds. Samples of seed were removed from the crucibles and were exposed to the stimulating solution immediately after washing. It would have been preferable to have kept the seeds in total darkness throughout the experimental period, but this was not possible. However, the period of exposure of the seeds to daylight was standardized at 5 minutes every 3 days of pretreatment.

The seeds of *Striga hermonthica* are relatively minute (0·4×0·2 mm.) and thus microscopical examination is necessary to determine germination. The method of exposing *Striga* seeds to the stimulating solution facilitated microscopic inspection but differed from that of Brown and Edwards (1944) in that it permitted a representative population of seeds to be examined. Small ointment pots with a capacity of 25 ml. were used as the germination vessels. The bottoms of these pots were concave and so before the seeds were placed inside it was necessary to prepare a platform on which the seeds could be sown. With this aim in view a few glass beads were placed in each pot. Two discs of 4·25 cm. filter-paper were laid on top of the beads, and 3 ml. of either the stimulating solution or distilled water were then added to each vessel. After this, a well-washed 4-cm. disc of permeable cellophane was placed on top of the filter-paper. The pots were then ready for use as germination vessels. At this stage 150–200 *Striga* seeds were spread evenly over the cellophane by means of a camel-hair brush.

After sowing, the ointment pots were sealed to prevent water loss. This was accomplished by fixing a disc of water-proof cellophane over the top of each pot and securing it to the lip of the pot by a rubber band. The ointment-pot lids were then replaced and held in position by rubber bands.

For germination counts the cellophane disc together with the attached seeds was removed, then placed on a grid scratched on a glass plate and examined under a binocular microscope.

All germination tests were replicated at least 3 times and all data were analysed for variance.

EXPERIMENTAL RESULTS

Preliminary observations

Preliminary experiments were first carried out in 1946 to determine whether germination could be induced by treating the dry seeds with the stimulating solution. It was found that no more than 0·1 per cent. of the seeds germinated when they were placed in vessels containing the stimulating solution and were then incubated at either 22° C. or 32° C. for varying periods up to 21 days. (During these experiments the water lost by evaporation was replaced every 3 days.) The seeds used in the preliminary experiments were harvested in 1940, 1941, and 1945 respectively. Consequently it seemed unlikely that the failure to induce more than a very small percentage of germination could be attributed to the use of immature seed.

The experiments so far described did not show whether the dry seeds were impermeable to the stimulant, whether the stimulant was having any action on the dry seeds, or whether the stimulant was effective in promoting some reaction in the seed, as a result of which subsequent germination was almost completely inhibited. Further experiments were clearly necessary to determine which, if any, of these three factors was involved when dry seeds were added to the stimulating solution. Additional observations suggested that the dry seeds were not impermeable to the stimulant and that the stimulant did have some very definite action on the seeds which almost entirely prevented subsequent germination. Thus when dry seeds were placed in germination vessels containing the stimulating solution and were transferred to fresh stimulating solution every 3 days only 0·1 per cent. germinated as before. On the other hand, when seeds harvested in 1940 and 1941 were given as little as 24 hours of moisture-treatment at a temperature of 22° C., and were then placed in germination vessels containing the stimulating solution, up to 10 per cent. germinated. It seemed therefore that a short period of moisture-treatment was essential if more than 0·1 per cent. of the seeds were to germinate.

These preliminary experiments showed that in common with the seeds of *Orobanche speciosa* (Chabrolin, 1938) and of *Striga lutea* (Brown and Edwards, 1944, 1946) the germination process of the seeds of *Striga hermonthica* involves two major phases. During the first phase, termed pretreatment, moisture only is required. During the second the stimulant is needed also. In view of the importance of the moisture-treatment, observations were made to determine how far it governs the amount of germination, using seeds of four different ages.

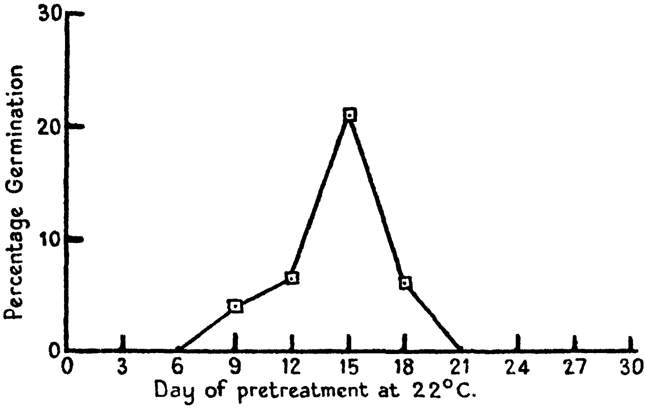

Fig. 1. The relationship between length of pretreatment at 22° C. and the germination capacity of 1945 seed. (Pretreatment initiated April 1946: standard error of each mean germination percentage = ±1·5.)

The effect of pretreatment and after-ripening on germination

When samples of 1945 seed were pretreated at 22° C. in April 1946, the germination capacity of the seed, after standard treatment with the stimulating solution, was found to vary with the length of pretreatment. The results of these experiments are shown in Fig. 1. Essentially similar results were obtained when 1948 seed was pretreated 4 months after harvesting. It may be seen from Fig. 1 that after 6 days of pretreatment at 22° C. germination was less than 1 per cent. From the 6th to the 15th day of pretreatment the percentage germination increased progressively and reached a peak value of only 21 per cent. after 15 days. During the subsequent period of pretreatment the percentage germination decreased, and after 21 days no more seeds germinated.

The low germination capacity, observed at the optimum stage of pretreatment in this experiment, was almost certainly due to immaturity of the seed employed. The results of additional studies on the germination of 1945 seeds, extending over a period of 15 months, confirmed this view. In these experiments samples of 1945 seed were pretreated at 22° C. prior to the application of the stimulant; but the experiments were separated by time-intervals varying from a few days to 6 months. A representative series of the germination curves obtained are shown in Fig. 2, which indicates also the month in which the pretreatment of each sample was initiated.

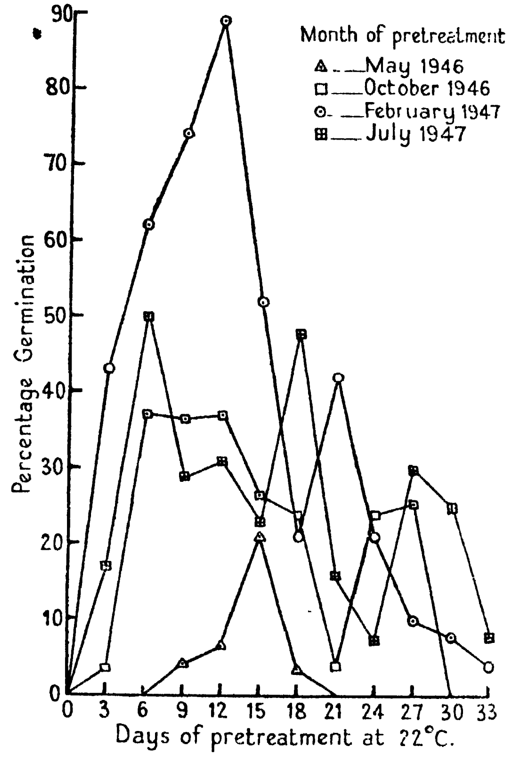

FIG. 2. The relationship between length of pretreatment at 22° C., the date of initiating pretreatment, and the germination of 1945 seeds.

From Fig. 2 it may be seen that whilst there were obvious differences in the curves of germination and in the maximum percentage of germination achieved in the four series, each was alike in showing a period of pretreatment to be necessary before the sample reached its maximum germination capacity. The data obtained from the experiments begun in April 1946, October 1946, and February 1947 might be taken as a clear indication that after-ripening does influence germination, for the peak germination percentage in each experiment was 21, 37, and 89 per cent. respectively. If this supposition is correct it would seem that after-ripening took place progressively during the time that the dry seed was stored in the laboratory.

The results of the germination study begun in July 1947 suggested that this explanation might not be valid, for as may be seen from Fig. 2, the germination curve showed two maxima and the peak germination was only 50 per cent. It seemed unlikely that the differences between the February curve and the July curve could be attributed to the loss of seed vitality, for a peak germination of 72 per cent. was obtained in further experiments on 1945 seed in November 1947. Thus the differences might have been due either to a further manifestation of after-ripening or, alternatively, to changed experimental conditions. Consideration of the data on the germination of 1945 seed alone might suggest that the variations described were due to some change in experimental conditions, particularly as reproducible results were only obtained when samples of seed were pretreated simultaneously. However, a comparative experiment on the effect of pretreatment on the germination of seeds harvested in three different years showed that after-ripening was probably the major factor responsible for the differences in the germination curves shown in Fig. 2.

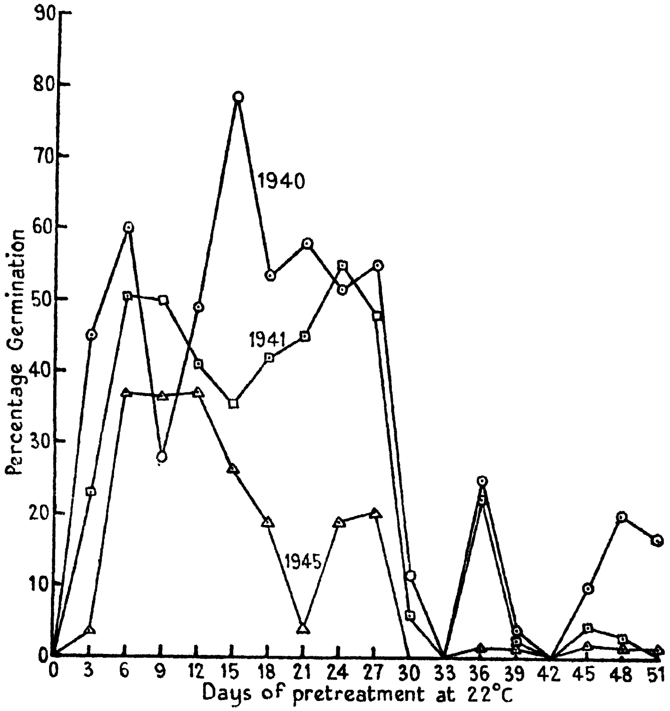

FIG. 3. The effect of length of pretreatment at 22° C. on the germination of seeds harvested in 1940, 1941, and 1945. (Pretreatment initiated October 1946: standard error of each mean germination percentage = ±3·6.)

Fig. 3 gives the data obtained by germinating samples of 1940, 1941, and 1945 seed at the same time by the standard method. This experiment was begun in October 1946 and the pretreatment of the three seed samples was initiated simultaneously. It will be observed from Fig. 3 that the germination capacity of the seeds of each sample increased during the first phase of pretreatment and that the respective peak germinations of the 1940, 1941, and 1945 seeds were 78, 58, and 37 per cent. The maximum germination percentages of the 1940 and 1941 seeds were higher than that of the 1945 seed in the replicate of the experiment illustrated by Fig. 3, but the results as a whole were significantly different from those shown in Fig. 3. However, additional experiments have indicated that the differences between the results shown in Fig. 3 and those of the replicate experiment could be attributed to the fact that in the replicate experiment the temperature exceeded 22° C. during the first 24 hours of pretreatment.

Now in these comparative experiments the seeds were all exposed to the same experimental conditions and consequently the differences between the peak germination capacities of the youngest and older seed samples cannot be ascribed to experimental error. Furthermore, the experiments illustrated in Fig. 2 show that in October 1946 at least 89 per cent. of the 1945 seeds were viable, whereas at the time of this experiment only 37 per cent. was induced to germinate together. In view of all these results it is considered that the germination of the seeds of *Striga hermonthica* is greatly influenced by after-ripening.

Certain additional experiments suggested that the rate of after-ripening is enhanced when dry seed is stored in the laboratory, as compared with the rate of the process in the native habitat. Thus when germination studies were carried out on 1940 and 1941 seeds in 1947, up to 42 per cent. of the seeds were induced to germinate in the absence of the stimulating solution. Furthermore, at this stage of after-ripening, the results of replicated experiments, separated by time-intervals of up to 7 days, did not differ significantly. The data obtained using 1940 seed in February 1947 are shown in Fig. 4; the data on the germination of 1941 seed in May 1947 are given in Fig. 5. As in previous experiments it will be seen that in the presence of the stimulating solution the amount of germination first increased as pretreatment was extended and then diminished after 27 days of pretreatment. It will be observed that in each figure the curve of germination, in the presence of the stimulating solution, is no longer cyclic and that the peak germinations of the 1940 and the 1941 seeds were 82 per cent.$\pm 3{\cdot}3$ and 63 per cent.$\pm 2{\cdot}3$ respectively. Both figures show a cyclic rise and fall in the percentage of spontaneous germination, but whereas with the 1940 seeds the maximum spontaneous germination was induced after 24 days of pretreatment; with the 1941 seeds this occurred after only 9 days of pretreatment. These differences were presumably due to the use of seed of different ages and not to the experimental technique. It must be emphasized, however, that, with one exception, spontaneous germination did not take place in the pretreatment vessels. It was induced only by incubating pretreated seeds, moistened with distilled water, for 48 hours at 32° C. In the exceptional case 5 per cent. of the seeds germinated spontaneously in the pretreatment vessels after 1940 seeds had been pretreated for 24 days at 22° C.

The effect of a second pretreatment on germination

It may be seen from Figs. 1 to 5 that after 15 days of pretreatment in the experiment illustrated in Fig. 1 and after 27 days in the other experiments the amount of germination diminished. In some experiments, such as those whose data are shown in Figs. 2 and 3, the percentage of germination at the end of the experimental period approached zero. Clearly it was desirable to ascertain whether pretreatment at 22° C., for the periods shown in Figs. 1 to 5, resulted in the death of the majority of the seeds, or alternatively whether the small amount of germination at the end of each experimental period was due to the seeds passing into a secondary state of dormancy towards the end of each experiment.

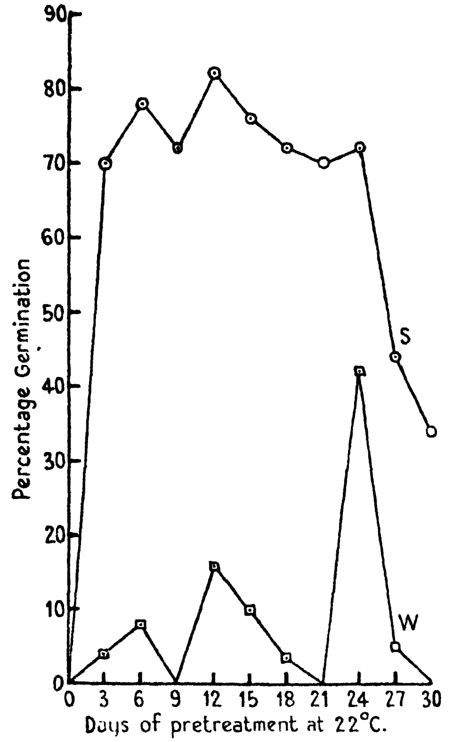

Fig. 4

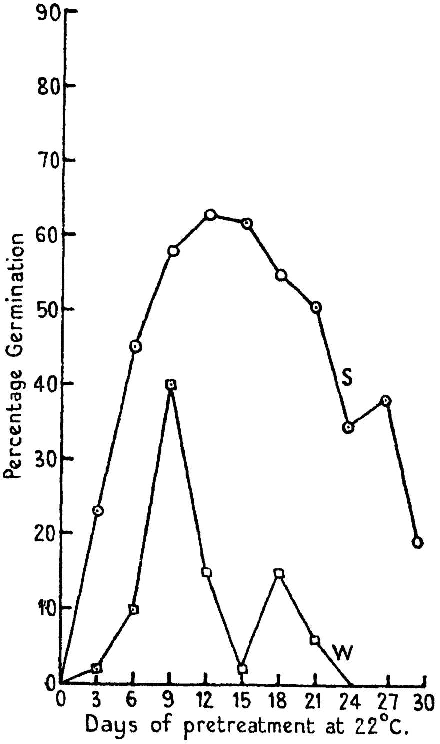

Fig. 5

Fig. 4. The effect of length of pretreatment at 22° C. on germination in the presence of the stimulating solution (s) and on germination in distilled water at 32° C. (w) 1940 seed. (Pretreatment initiated February 1947: standard error of each mean germination percentage = ±3·3.)

Fig. 5. The effect of length of pretreatment at 22° C. on germination in the presence of the stimulating solution (s) and on germination in distilled water at 32° C. (w). 1941 seed. (Pretreatment initiated May 1947: standard error of each mean germination percentage = ±2·3.)

Brown and Edwards (1944) showed in the case of the seeds of *Striga lutea* that if the temperature of pretreatment was increased at the time when the germination capacity of the seeds was falling, the germination capacity was enhanced compared with that at the initial temperature of pretreatment. This method was without effect on the germination of the seeds of *Striga hermonthica.* However, the loss of seed vitality during pretreatment in the experiments illustrated by Figs. 1 and 3 was not higher than 50 per cent. in the oldest seed and was much less than this in the 1945 seed. This was demonstrated by drying the seed at the end of each experiment and applying the germination technique to the dried seeds. Fig. 6 shows the relationship between the germination of seeds harvested in 1940, 1941, and 1945 and the length of the second

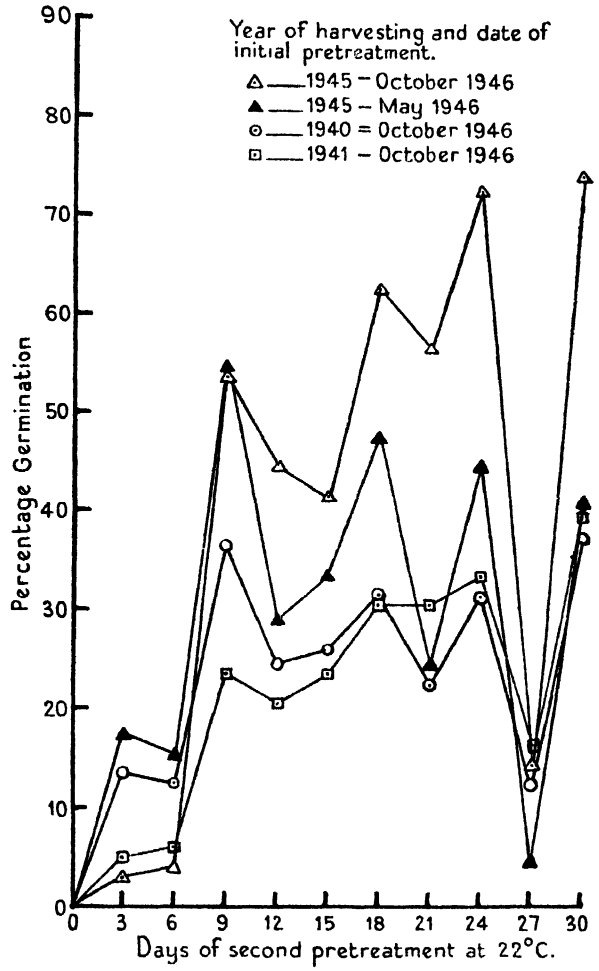

FIG. 6. The effect of length of a second pretreatment at 22° C. on the germination of seeds harvested in 1940, 1941, and 1945 respectively. For details see text. (Second pretreatment initiated in November 1946: standard error of each mean germination percentage = ±2·0.)

pretreatment. Each point plotted in the figure is the mean percentage germination of six replicates. The data shown in Fig. 6 were obtained by drying the seeds remaining at the end of the experiments illustrated by Figs. 1 and 3 for 48 hours over concentrated sulphuric acid and then applying the germination technique to the dried seeds. The second pretreatments at 22° C. of the four seed samples were initiated simultaneously in November 1946.

It may be seen from Fig. 6 that the peak germination percentages of the 1945 seeds were higher than those in the initial experiments. Thus during the first pretreatment the peak germination was 21 per cent. when pretreatment was begun in April, and 37 per cent. when begun in October. The peak germinations obtained as a result of the second pretreatment were 52 and 74 per cent. respectively.

The differences in the maximum germination capacities of the samples of 1945 seed during the second pretreatment at 22° C. cannot be explained at this stage of the investigation. Nevertheless, it is clear that many of the seeds became dormant once more during pretreatment, in the experiments illustrated by Figs. 1 and 3.

An initial pretreatment at a temperature exceeding 22° C. was found to reduce markedly the capacity of the seeds to germinate after a second period of pretreatment at 22° C. Thus when seeds harvested in 1940 and 1945 were pretreated for 27 days at 27° C. and at 32° C., the maximum germination observed, after drying followed by pretreatment at 22° C. and exposure to the stimulating solution, was 10 per cent.

The relationship between temperature of pretreatment and germination

Various early experiments suggested that if seeds were pretreated at a higher temperature than 22° C. the effect of this would be not merely to shorten the period of pretreatment required by the seeds to enable them to reach their maximum germination capacity, compared with the period at 22° C., but to reduce their germination capacity. To determine the relationship between temperature of pretreatment and germination two comparative experiments were carried out simultaneously. In these experiments seeds harvested in 1940 and 1945 were pretreated at four different temperatures, namely 12° C., 22° C., 27° C., and 32° C. Two samples of each year's seed were pretreated at each temperature and three replicate lots of seed from each pretreatment vessel were exposed to the stimulating solution at times separated by 3-day intervals.

Fig. 7 shows the effect of pretreatment at the four different temperatures on the germination of seeds harvested in 1940. Pretreatment in this experiment was begun in November 1946. Statistical analysis of the data given in Fig. 7 showed that the differences in the germination percentages of the replicates and those between the germinations of the two seed samples were not significant.

In this series it will be seen that where seeds were first pretreated at 32° C. and 27° C. and then incubated for 48 hours in germination vessels containing the stimulating solution, the maximum amount of germination was less than that induced after a period of pretreatment at 22° C. The maximum percentage germinations were 22, 56, and 81 per cent. respectively. In addition to this it will be observed that the germination capacities of seeds pretreated at either 27° C. or 32° C. were never higher at any one stage than the comparable germination capacity of seed pretreated at 22° C. Furthermore, no more seeds germinated after 24 days of pretreatment at either 27° C. or 32° C. Temperatures of pretreatment exceeding 22° C. had a similar effect on the germination capacity of 1945 seed.

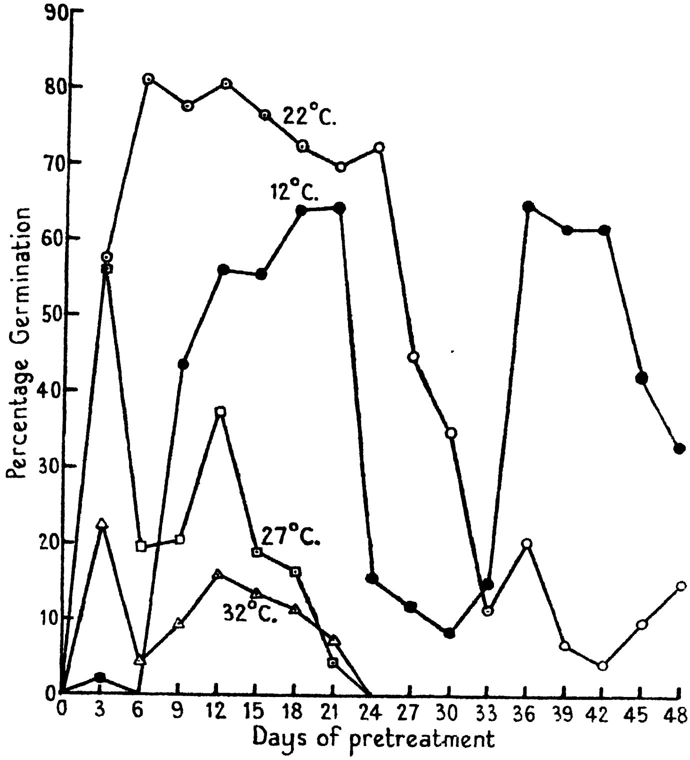

FIG. 7. The relationship between length of pretreatment, temperature of pretreatment (12° C., 22° C., 27° C., and 32° C.), and the germination of 1940 seed. (Pretreatment initiated November 1946: standard error of each mean germination percentage = ±3 8.)

Additional experiments on seeds harvested in 1940 and 1945 have shown that if seeds were pretreated at temperatures exceeding 22° C. for as little as 3 days, and were pretreated from then on at 22° C., the germination curve resembled that given after pretreatment for the whole period at the initial temperature. Thus whatever this temperature effect may be, it seems that the seeds do not recover from it during subsequent pretreatment.

The germination data obtained by exposing seeds pretreated at 12° C. to the stimulating solution shows certain features of considerable interest. It may be seen from Fig. 7 that during pretreatment at 12° C. the germination capacity first increased to a peak value of 65 per cent., diminished to less than 10 per cent., and then rose once more to 65 per cent. These data suggest that some of the seeds were in a state of secondary dormancy from the 21st to the 33rd days of pretreatment. Further pretreatment broke this dormancy so that after 36 days 65 per cent. of the seeds were able once more to germinate.

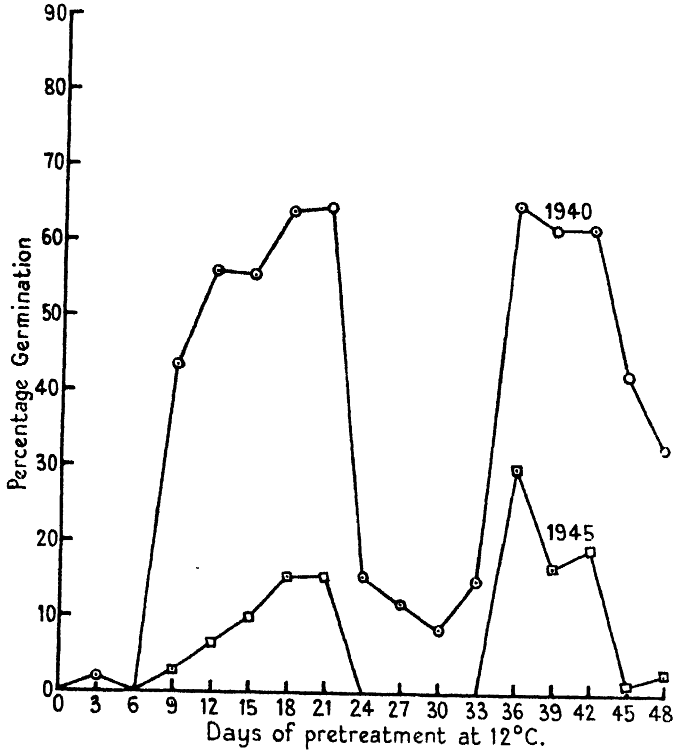

FIG. 8. The relationship between length of pretreatment at 12° C., the age of the seed (1940 and 1945), and the germination capacity. (Pretreatment initiated November 1946: standard error of each mean germination percentage = ±3·8.)

Essentially the same type of germination curve was obtained when 1945 seeds were pretreated at 12° C. Fig. 8 shows the data of the germination capacity of 1945 seed during pretreatment at 12° C. The comparable curve of 1940 seed, taken from Fig. 7, is included in Fig. 8. Subsequent experiments have shown that a curve of germination capacity with two maxima is always obtained when seeds are pretreated at temperatures from 12° C. to 20° C. and the germination data appropriately plotted. Thus whereas the characteristics of the various germination curves, drawn from the germination data of seeds pretreated at 22° C., have differed considerably, those curves derived by plotting the germination capacity during pretreatment at temperatures from 12° C. to 20° C. always showed certain similar features.

The effect on germination of diluting the standard stimulating solution

The relationship between germination and dilution of the stimulating solution was found to be influenced by two factors, namely, the age of the seed and the amount of pretreatment. This was shown by two experiments. In the first 1945 seed was pretreated at 22° C. from the end of May 1946, and the pretreated seed was incubated for 48 hours at 32° C. in germination vessels containing various dilutions of the stimulating solution. In the second experiment seeds harvested in 1940 were treated similarly. The pretreatment of this seed was begun in February 1947.

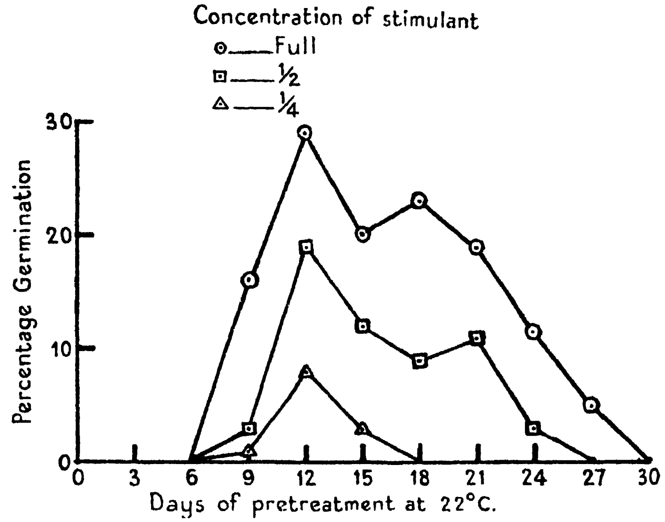

FIG. 9. The relationship between the length of pretreatment at 22° C., the concentration of the stimulating solution, and the germination of 1945 seed. (Pretreatment initiated May 1946: standard error of each mean germination percentage = ±1·8.)

Fig. 9 shows the effect of various dilutions of the stimulating solution on the germination of 1945 seeds after periods of up to 30 days of pretreatment. In this experiment glass-distilled water was added to the standard solution of the stimulant to give dilutions of 1:1 and 1:3. Two samples of seed were pretreated at 22° C., and at 3-day intervals seeds were withdrawn from the pretreatment vessels, and three replicate lots of seed from each sample were incubated for 48 hours in contact with each dilution of the stimulating solution. Each point plotted therefore represents the average value of six determinations of germination percentage. An Analysis of Variance showed the effect of stimulant-dilution to be highly significant, whereas the differences between seed samples and replicates were not significant.

It will be seen from Fig. 9 that between the 9th and 27th days of pretreatment the germination induced by treating the seed with the half-strength stimulating solution was lower than that resulting from the exposure of the seeds to the standard solution. Furthermore, the ratio between the germination induced by the standard solution and that by the half-strength solution was not constant during pretreatment. This ratio was 0·19, 0·65, 0·60, 0·39, 0·57, and 0·27 at the 9th, 12th, 15th, 18th, 21st, and 24th days of pretreatment respectively.

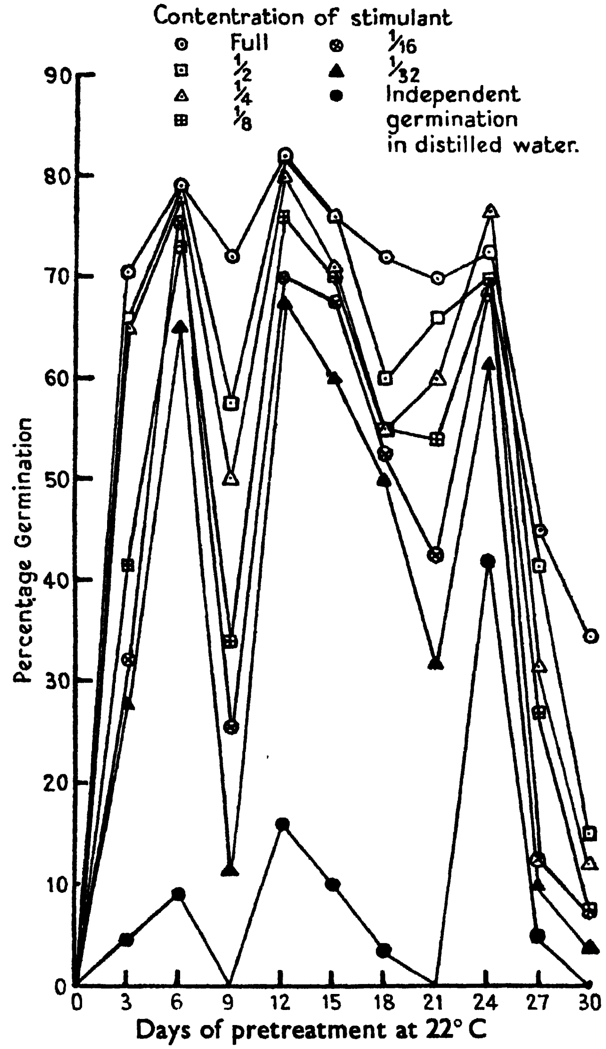

FIG. 10. The effect of dilution of the stimulating solution and length of pretreatment at 22° C. on the germination of seeds harvested in 1940. (Pretreatment initiated February 1947: standard error of each mean germination percentage = ±3·3.)

When 1940 seeds were used in a similar experiment begun in February 1947, the stimulating solution was further diluted. In all, dilutions of the stimulating solution of 1:1, 1:3, 1:7, 1:15, and 1:31 were employed, together with the standard solution and a control series with distilled water. The data of this experiment are given in Fig. 10. The Analysis of Variance of this data showed that the differences between the amounts of germination of the two samples of seed when exposed to either the standard solution or to distilled water were not significantly different. On the other hand, whereas the replicates did not differ significantly, the amounts of germination of seeds from the two samples after other treatments did differ significantly. However, as the dilution effects always showed a similar trend, the two sets of data were combined. Each point plotted in Fig. 10, therefore, is the mean germination percentage of two series each of three replicates.

The dilution effects shown in Fig. 10 are very striking and it will be immediately apparent that they are very different from those shown in Fig. 9. It may be seen from Fig. 10 that stimulant-dilution was least effective in reducing the germination at each of the peak phases of independent germination. At these times, namely the 6th, 12th, and 24th days of pretreatment, the germination was not reduced by dilutions above 1:15, 1:7, and 1:15 respectively. At other times, such as the 9th and 21st days, the effect of dilution on germination was considerable.

The nature of these effects is at present obscure. If, as suggested by Brown and Edwards (1946), in the case of *Striga lutea*, the seed manufactures the stimulant itself at the time of peak independent germination, it would mean that with *Striga hermonthica* the stimulant is progressively formed and destroyed as pretreatment proceeds. This may seem unlikely, but insufficient evidence is available at present either to confirm or refute this hypothesis.

Discussion

It will be clear from the work described that a period of pretreatment followed by a relatively short period of exposure to a suitable stimulating solution is necessary before the optimum germination of the seeds of *Striga hermonthica* will take place. The two processes, though complementary, seem to be independent, for within the experimental limits the optimum temperature for pretreatment was 22° C., whilst that for treatment with the stimulating solution was 32° C.

The variation in the germination curves, obtained by plotting the germination capacities of the seeds in the presence of the stimulating solution during pretreatment at 22° C., could possibly be ascribed to one or more of three main factors. These are: (*a*) differences between samples of the experimental material due to the use of a small seed population, (*b*) variation in the conditions of pretreatment and in the conditions of the treatment with the stimulating solution, and (*c*) differences in the state of the dry seed immediately prior to the initiation of pretreatment.

It is thought that the seed population used in the pretreatment vessels was large enough to be fully representative, for 0·5 g. of *Striga hermonthica* seed contains at least 40,000 seeds, and hence this was neglected as a determining factor. The conditions of pretreatment and exposure to the stimulant also cannot be the major factor in determining the differences, for in the comparative experiments carried out on 1940, 1941, and 1945 seed the peak germination capacity of the older seed samples was always higher than that of the 1945 seed whether the pretreatment temperature was 12° C. or 22° C.

All things considered, it seems that the major factor responsible for the variation in the curves of germination frequency after pretreatment at 22° C. must be the condition of the seed immediately prior to the initiation of pretreatment, or in other words the stage of after-ripening. This after-ripening process must be of long duration when the seed is stored dry under tropical conditions, as comparatively high germination in the absence of the stimulating solution was only brought about in January 1947 in the case of 1940 seed and in May with 1941 seed, that is, after at least 6 years of storage. The changes which took place in the capacity for independent germination during the storage period in this laboratory would also indicate that after-ripening proceeds more readily at temperatures lower than those normally encountered in the native habitat.

It would be premature to attempt to interpret much of the experimental data presented at this stage of the investigation. However, the problems raised have suggested that a study of the drift of respiration, both during pretreatment and whilst the seed is in contact with the stimulating solution, would be of great value in elucidating some aspects of the germination process. It is hoped to publish an account of such work in the near future.

SUMMARY

1. The optimum germination of the seeds of *Striga hermonthica* will not be realized unless they are exposed to a suitable stimulating solution.

2. Data are presented which show that a period of exposure to moisture is necessary before the seeds are placed in contact with the stimulating solution if more than 0·1 per cent. are to germinate. Following the terminology of Brown and Edwards (1944) this moisture-treatment has been termed 'pretreatment'.

3. From germination curves, obtained by plotting germination percentage against length of pretreatment at 22° C., it has been shown that after-ripening has a considerable influence on the germination of the seeds of *Striga hermonthica*. Studies on seed of four different ages have shown that the peak germination capacity increases as the seed ages and that the shape of the curve of germination is influenced by the age of the seed.

4. The capacity of the seed to germinate in the absence of the stimulant was found to increase with age. When seeds harvested in 1940 and 1941 were pretreated in February and May 1947 respectively, 42 per cent. germinated in the absence of the stimulating solution.

5. When pretreatment at 22° C. was prolonged beyond a certain limit the germination capacity of the seeds approached zero. It is considered that this was due, except when the seeds were almost fully ripe, to the seeds passing into a condition of wet dormancy. It has been shown that this secondary dormancy could be overcome by drying the seed and applying the germination technique once more.

6. Pretreatment at temperatures of 27° C. and 32° C. reduced the peak germination capacity compared with that determined after pretreatment at 22° C.

7. The germination curve obtained by applying the standard germination technique to seed pretreated at 12° C. showed two maxima irrespective of seed age.

8. The relationship between length of pretreatment, germination percentage, and dilution of the stimulating solution was found to vary with the age of the seed. When 1945 seed was used 6 months after harvesting, it was found that dilution of the stimulating solution reduced the germination, compared with that induced by the standard solution. In experiments in which seed harvested in 1940 was employed it was found that, compared with the standard solution, a dilution of the stimulating solution of 1:31 reduced the germination. The effect of dilutions of 1:1, 1:3, 1:7, 1:15, and 1:31 was found to vary with the stage of pretreatment, but dilution had the least effect at the 6th, 12th, and 24th days of pretreatment. These times corresponded with those of the peak phases of independent germination.

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Observations on the Biology of *Fomes annosus*, with Particular Reference to East Anglian Pine Plantations

I. The Outbreaks of Disease and Ecological Status of the Fungus

BY

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With Plates XII and XIII and one Figure in the Text

INTRODUCTION

A ROOT-DISEASE of pines (*Pinus sylvestris* L. and *P. nigra var. calabrica* Schneid.) has recently caused serious damage in some plantations established by the Forestry Commission in East Anglia. Macdonald (1939) first drew attention to these outbreaks, which were then confined to young trees planted on former woodland sites. From about 1941 onwards there was a more serious development of the disease, which killed considerable numbers of 20-year-old pines, chiefly on former arable land. Research into the biology of *Fomes annosus* Fr., which was apparently the causal fungus, was started in September 1945, at the suggestion of Professor F. T. Brooks, F.R.S. This paper gives a brief summary of the existing information about *F. annosus*, describes the characteristic features of the East Anglian outbreaks, and discusses the status and general biology of the fungus. An earlier paper (Rishbeth, 1949) is concerned with the implications of the disease for forestry practice.

SUMMARY OF EXISTING INFORMATION ABOUT *F. ANNOSUS*

Fomes annosus is recorded as a frequent cause of root and butt rot in conifers in the northern hemisphere. Serious outbreaks of disease have occurred, for instance, in India, Russia, Norway, Bosnia, France, and the U.S.A. Although the distinction is not always made in the literature, it appears that the killing of young conifers by the fungus is also fairly widespread. Thus Rennerfelt (1947) records that 20–30-year-old pine stands in south-west Sweden were badly attacked. Bornebusch and Holm (1934) refer to the death of young conifers, including pines, following the replanting of a former spruce plantation in Denmark. Serious losses of pines have been reported from France, particularly the central plateau and the Landes and Sologne districts, and also from Belgium and Germany. More recently, Wagener and Cave (1946) have described the killing of 200-year-old pines (chiefly *Pinus jeffreyi* and *P. ponderosa*) in a dry, sandy region of California. One of the few records of a similar nature from the British Isles is given by Anderson (1924), who described the

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dying of groups of pines in Northumberland and Lanarkshire. Although *F. annosus* is widespread as an agent of butt rot (Peace, 1938), it is apparent that the outbreak in East Anglia is the first of its kind on any considerable scale in this country.

Much cultural work has been carried out on *F. annosus.* Cartwright and Findlay (1934) give the growth rates on malt agar at various temperatures: the maximum is at about 23° C., when the daily increase in diameter of a Petri-dish culture is 13 mm. Weis and Neilsen (1927) found that the most favourable hydrogen-ion concentration for growth on malt agar lay between pH 4·0 and 4·5. Rennerfelt (1944) found that growth in a liquid medium containing purified salts and sugar was very poor in the absence of thiamin. Extracts of humus or compost gave marked increases in growth. *F. annosus* has been found to produce no less than sixteen enzymes (Miller, 1943). Since cellulose is decomposed in addition to lignin, a typical white rot is produced in wood. No worker has been able to obtain growth of the fungus on forest humus or soil unless this was previously heat-treated. Hiley (1919) obtained growth from mycelium and spores on sterilized larch twigs, blocks of larch wood, roots of larch and Weymouth pine, and various soils. He attributed the failure to grow on unsterilized soils to inhibition by bacteria. Treschow (1941) found that no growth was obtained on a soil which had been sterilized and then inoculated with *Trichoderma* sp., whereas vigorous growth followed on soil similarly inoculated with *Bacillus subtilis* or *Actinomyces* sp. Despite these findings, many workers, including Falck (1930) and Rennerfelt (1944), state that the fungus spreads from tree to tree in the humus layer.

Most previous investigations on the parasitic activities of *F. annosus* have been concerned with the problem of butt rot. There has been a considerable divergence of opinion on the mode of infection. Hartig (1894), who carried out the earliest important work on the fungus, stated that spores are brushed off on to roots by burrowing rodents, and that germination of the spores is followed by penetration of the cortex. Further infections were believed to take place by growth of the fungus from root to root where these are in contact or have fused. Huet (1936) made a similar diagnosis for severe outbreaks in Belgium. Lagerberg (1936), however, stated that infection of spruces can only take place through dead roots. Anderson (1924) thought it probable that roots are weakened by the diffusion of toxic substances from decaying stumps and that infection then follows. Růžička (1928) believed that invasion by *F. annosus* follows asphyxiation of the roots caused by excessive compactness of the soil, waterlogging, or a coarse humus covering on the surface. Rhoads and Wright (1946) thought it probable that entry occurs through wounds above ground level in *Tsuga heterophylla.*

In two instances recently the killing of pines has been specifically mentioned. Wagener and Cave (1946) found that, in California, *F. annosus* does not require wounds or dead tissues before the root is invaded. Day (1946, 1948) similarly concluded that, in East Anglian plantations, *F. annosus* can infect living tissues which have received no mechanical injury, but he suggested that invasion by the fungus is secondary to severe injury of the root system brought about by adverse physical conditions of drought or waterlogging.

It is hardly surprising that a wide variety of soil conditions has been associated with outbreaks of root and butt rot. Different conifers may vary greatly in their resistance to *F. annosus* on a given type of soil, as may a single species on a range of soil types. Resistance may also be affected by climate and other factors. Many workers agree, however, that the disease is likely to be severe in plantations established on former arable land. The prevalence of disease on old woodland sites is also frequently mentioned, but it has only once been clearly associated with the previous thinning of a plantation (Hepting and Downs, 1944). The conditions associated with the killing of trees by *F. annosus* have received little attention.

THE OUTBREAKS OF DISEASE

(i) *The affected areas*

The disease has developed in two main areas. The first, comprising the Thetford and Swaffham forests, lies for the most part in the area known as Breckland, that is, in north-west Suffolk and south-west Norfolk. Owing to the wide variety of diseased plantations and the comparative nearness to Cambridge, most of the work was done here. The second area, comprising the Rendlesham and Tunstall forests, lies a few miles from the coast of east Suffolk.

In both areas the rainfall is low, the average annual figure for the last 10 years being 21 in. The mean monthly temperatures for the same period range from 3° C. (January) to 17° C. (July). In Breckland the soil is derived from a chalky glacial drift and interglacial sands, the underlying formation being chalk. Large areas are covered by wind-blown sand. The soils are uniformly sandy, but show considerable variation chemically: where the chalk is near the surface the calcium carbonate content and pH value are high, but in other areas the base status and pH value are low and well-developed podzols may be present. The natural vegetation is largely grassland with societies of *Pteridium aquilinum* (L.) Kuhn and *Carex arenaria* L., but considerable stretches of heath are dominated by *Calluna vulgaris* (L.) Hull. Much derelict arable land is also present. In east Suffolk, on the other hand, the soil is derived from a sandy or gravelly drift beneath which is Norwich Crag. With the exception of land formerly cultivated, the soils are very acid. *Calluna vulgaris* is dominant over nearly the whole area, and grows more luxuriantly than in Breckland.

(ii) *The recent history of the areas*

Since the development of disease in the plantations is influenced by the former status of the sites, it will be convenient to refer briefly to the recent history of the affected areas. In the early part of the nineteenth century much of Breckland was cultivated for the first time. Associated with this, many windbreaks were established, the commonest type being a single line of *Pinus* *sylvestris*, but larger shelter strips comprising mixtures of conifers and deciduous trees were also planted. There were few large woods before 1840 and the trees in them were predominantly deciduous. Later in the century, however, several plantations were established in which the bulk of the trees were conifers. Large areas of grassland and heath still remained uncultivated, and in succeeding years much of the arable land became derelict. In the Rendlesham forest area only a few small woods and shelter belts existed before 1900.

From 1921 onwards the Forestry Commission established plantations on all types of land, the total area planted in Breckland being about 50,000 acres. Although records are incomplete, it is estimated that the areas planted on former woodland, heath (including grassland), and arable are in the approximate ratio of 1:3:1. The majority of former woodland sites were replanted from 2 to 4 years after felling, although occasionally a much longer period elapsed. Shelter strips adjacent to arable or heath sites were felled either before planting or at a variable time afterwards. In addition to planting, the Forestry Commission acquired a number of woods which had been established before 1922. *Pinus sylvestris* is the tree most commonly planted, and, after that, *P. nigra* var. *calabrica*. To a lesser extent *Pseudotsuga taxifolia* (Poir.) Britt., *Larix decidua* Mill., and a number of other species occur.

(iii) *The sources of infection*

Root disease has killed young trees on former woodland sites right from the beginning of replanting. Areas replanted after fire also show disease at an early stage. Deaths often occur 2 years after planting and continue for a variable period, sometimes as long as 19 years. In such plantations it is common to find groups of dead trees, around which each year one or two more lose vigour and die. By contrast, disease has appeared in plantations established on former arable or heath chiefly after thinning. In such sites there are also many instances of trees dying at the edge of felled woodland strips or along the border of 'racks' cut for access, or of 'rides' cut for fire protection. The most devastating attacks, however, have all followed thinning carried out in plantations about 15 years old. Much of this was of the 'pre-thinning' type, where relatively few coarsely growing trees were taken out, but there were also experimental thinnings, some of which were very heavy. As with plantations on former woodland, the trees characteristically die in groups, often circular, and this may lead to the formation of gaps up to 25 metres across.

In every site where the disease has developed, *Fomes annosus* is present in stumps. The source of infection is usually near the centre of gaps caused by the disease. Stumps in such places do not invariably contain *F. annosus*, but the likelihood of its former presence in them is suggested by the type of rot and by the fact that similar stumps in the area still contain the fungus. In every site containing infected stumps, moreover, it is possible to find living roots actively invaded by *F. annosus*.

In nearly half of the seventy sites investigated, infection has arisen from stumps of the previous crop. Of the remaining sites, where infection followed thinning in, or felling near, the standing crop, over a half were formerly arable. By contrast, only one-tenth of the remaining sites were formerly *Calluna* heath. This is partly because early thinning is infrequent in plantations on such sites and also because, when thinned, they suffer far less damage than similar plantations on former arable. Serious outbreaks of disease, such as those occurring on former arable, are comparatively recent because thinning was not carried out before 1937 and was widespread only from about 1942 onwards.

(iv) *The symptoms of disease*

The symptoms of disease and severity of attack are very similar in *Pinus sylvestris* and *P. nigra* var. *calabrica.* In the following account no distinction is made between the two species unless otherwise stated. In affected trees above 15 years of age the growth rate usually falls off for a year or two before death, and the crown shows various abnormalities: only one year's growth of needles is borne, or the needles themselves are unusually short. By the time these symptoms appear the root system is extensively invaded by the fungus. Resin may exude copiously from the roots, which often have characteristic paper-like sheets of mycelium between the bark-scales (Pl. XII, A). Before the crown dies, sporophores of *F. annosus* may appear at the base of the trunk in sheltered situations. The bark-beetle *Myelophilus piniperda* L. frequently invades the trunk either after death or when the root system is heavily infected; in the latter condition, symptoms of disease may still be lacking in the crown. This invasion may well hasten the death of the tree and create the opportunity for wood-rotting fungi such as *Polystictus abietinus* (Dicks.) Fr. and *Pleurotus mitis* (Pers.) Berk. to enter. Sporophores of these fungi were occasionally present some way up the trunk while the crown was alive. The early invasion by bark-beetles helps to explain the very rapid rotting of the trunks of trees killed by this root disease; in the absence of sporophores of *F. annosus*, the deaths might easily be attributed to bark-beetles, as has happened in the U.S.A. (Wagener and Cave, 1946).

(v) *The isolation of* F. annosus

Evidence that *Fomes annosus* is the cause of this type of attack was chiefly derived from a detailed examination of root systems. Chips of bark and wood from diseased roots were dipped momentarily in alcohol, surface-sterilized by immersion for 5 minutes in a solution of calcium hypochlorite (about 2 per cent. free chlorine), and then plated on malt agar. Cultures were incubated for 10 days at 22·5° C. When it was required merely to demonstrate the presence or absence of *F. annosus* in wood samples these were cleaned, wrapped in newspaper, thoroughly wetted with tap-water, and kept in glass containers at room temperature (17°–20° C.). Under such conditions the presence of *F. annosus* was indicated by the production of conidia, usually after 4–5 days, but only after 8–10 days when the tissues were resinous. These conidia are borne as characteristic heads (Pl. XIII, A and B), as originally described by Brefeld (1889), and are easily recognizable under the low power of the microscope. Since the conidia cannot be confused with those of any other common wood-inhabiting fungus, this direct incubation of wood or bark gives reliable results, and far fewer isolations on laboratory media are required. The proportion of positive results obtained by direct incubation is often greater than that obtained by plating, partly because *F. annosus* is somewhat irregularly distributed in the tissues, but also because saprophytic fungi grow freely on malt agar and often obliterate the slower growing parasite. The method was also used for large diseased roots. These were cut at selected places to expose the bark and wood, divided into suitable lengths, and then split longitudinally. After incubation, conidia indicated the approximate distribution of *F. annosus* in the bark and wood.

Trees recently affected by root disease almost invariably contained *F. annosus* in some part of the root system. The only exception was with trees affected by *Armilaria mellea* (Vahl.) Fr., which, however, was confined to sites previously bearing hardwoods or a mixed crop. The species of *Phytophthora* isolated from the diseased trees by Day (1946) was not found, nor were any other fungi of pathogenic significance. *F. annosus* was isolated in pure culture from the wood of pine roots which had recently been killed, although the bark sometimes had a superficial growth of other fungi, such as *Trichoderma viride* Pers. ex Fr., *Cylindrocarpon radicicola* Wollenw., and *Pencillium* spp. Such fungi also commonly occurred on the surface of healthy roots. In affected roots the boundary between healthy and diseased tissues, frequently marked by resin exudation, regularly contained *F. annosus*. The same fungus was also isolated from roots in the earliest stages of disease, when the affected portions were only a few centimetres in length. These observations strongly suggest that *F. annosus* was the causal parasite.

(vi) *The mode of infection*

Investigation of early stages of infection showed that, in every instance, the fungus had invaded a living root at a point where it touched another already infected. The first trees to be killed are always infected from stump roots (Pl. XIII, C), but such trees may in turn transmit the disease to adjacent ones. Infection occurs either on one or more healthy lateral roots at some distance from the bole, or at the bole itself. Stump roots never infect living roots unless they are in intimate contact with them. This, however, is not an important limitation to the spread of disease, since root contacts are frequent in plantations from about the eighth year onwards, and actual root fusions are moderately common. Later stages of infection are more difficult to interpret, but the source of infection can usually be found. The observation that initial attack by *F. annosus* is confined to the root system agrees with most previous work, but the part played by root contact in the spread of infection has not always been clearly stated.

THE ECOLOGICAL STATUS OF *F. ANNOSUS*

(i) *Habitat and host-range*

In East Anglia *Fomes annosus* is one of the commonest wood-rotting fungi present in coniferous stumps. During the investigation it was found in stumps of *Pinus sylvestris* L., *P. nigra* var. *calabrica* Schneid., *Pseudotsuga taxifolia* (Poir.) Britt., *Larix decidua* Mill., and *Picea abies* (L.) Karst. Parasitic attacks on all these species were seen and also on young *Chamaecyparis Lawsoniana* Parlatore. *F. annosus* was frequently present in stumps of *Betula pendula* Roth., but only occasionally in those of *Quercus robur* L. *Betula* showed a remarkable ability to survive attacks by the fungus: stumps with a considerable amount of rot often produced healthy shoots, and trees with a high proportion of rotted roots appeared normally healthy, though occasionally suffering wind-throw. *F. annosus* was isolated from the dying roots of 30-year-old *Quercus robur* L., *Fagus sylvatica* L., and *Populus italica* Moench., which were growing in a former shelter strip and were surrounded by numerous infected pine stumps. Since nearby trees, already dead, also contained *F. annosus*, it is probable that the fungus can kill these hardwoods under certain conditions. *Fagus*, without doubt, is killed sporadically soon after planting in some areas where infected stumps are present. Parasitic attacks on *Crataegus monogyna* Jacq. were also seen.

Sporophores of *F. annosus* were sometimes found on the stumps of *Ulex europaeus* L. cut out during clearing, and in one instance young pines near by had been infected from this source. The last and most surprising record is of *F. annosus* on *Pteridium aquilinum* (L.) Kuhn, on which it has been found four times. In one instance a rhizome had been infected by contact with a pine root, and the fungus was growing in the stelar tissues. In another a small sporophore was borne on the stump of a rotted frond. Tests made in the laboratory suggest that the fungus is unlikely to grow vigorously enough on bracken rhizomes to be a serious source of infection to trees. Attacks by *F. annosus* on the two hosts just mentioned have not previously been recorded, as far as is known.

In addition to these records from roots or stumps, *F. annosus* was occasionally found in sawn pine logs lying on the ground. A small amount of free mycelium was seen twice on litter and once on the floor of a vole tunnel, in each instance immediately beneath a sporophore of the fungus. Similarly, the only soil sample (out of about 250 tested) which produced colonies of *F. annosus* after plating a suspension of it in Dox's agar was obtained from beneath a sporophore. In other situations visible mycelium on the soil or litter always belonged to other fungi. No rhizomorphs of *F. annosus*, similar to those described by Miller (1943), were seen during the investigation; rhizomorphs present on the surface of roots rotted by *F. annosus* were found to belong to other basidiomycetes.

It is well known that the fungus may remain for years as an agent of decay in the stumps of trees which it has killed, but the means by which it colonizes the stumps of freshly felled trees has not been elucidated. Most workers have assumed either that it is already present in a dormant condition in the root system or that it enters from the soil or litter when the resistance of the tree is lowered by felling. The observations so far given throw little light on the problem, for it is notoriously difficult to isolate basidiomycetes from soil or litter by plating, and negative results are therefore not necessarily significant. Although, as described above, free mycelium of *F. annosus* was seen on litter, its distribution was so limited that little significance can be attributed to the observation.

(ii) *The growth of* F. annosus *on natural substrates*

If *F. annosus* can live saprophytically in soil or decomposing litter as well as in stumps, the potential source of infection to living trees is very great and, in some circumstances, invasion through mycorrhizal roots might take place. In view of the conflicting opinions about this in the literature, further evidence was sought.

In one experiment the ability of *F. annosus* to grow on freshly gathered samples of soil and litter from Breckland plantations was determined. To prepare the inocula, pine roots were cut into 5-cm. lengths, sterilized by autoclaving, and infected with agar inocula of the fungus. The roots, incubated at 22·5° C. in wide-necked stoppered bottles, were ready to use after 3 to 4 weeks. Sterile Petri dishes were half-filled with the soil to be tested, the soil surface was levelled, and a single inoculum of the type described above placed in the centre. If the soil was dry, it was brought to a moisture content of about 10 per cent. by the addition of sterile distilled water. The Petri dishes were placed in containers, to limit evaporation, and were incubated at 22·5° C. for 14 days. When the soil samples were previously sterilized by autoclaving, abundant growth from the inoculum always occurred but, with unsterilized samples, results were variable. With the latter the growth of mycelium from the inoculum was measured; the results, which are given in Table I, are grouped for convenience.

TABLE I

The Growth of F. annosus *on Unsterilized Soil and Litter from Breckland Plantations*

Description of sample.	Depth of sampling. (cm.).	Total no. of samples.	No. of samples giving growth (mm.) from the inoculum:			
			0.	1–2.	3–5.	>6.
Litter	0–1	16	14	2	0	0
Humus . . .	1	17	17	0	0	0
Acid soil . . .	8	13	6	4	1	2
„ „ . . .	15–30	18	6	4	1	7
Alkaline soil . .	8	18	7	1	6	4
„ „ . . .	15–30	20	5	1	3	11

It is clear that, under these conditions, growth of *F. annosus* in the humus layer or litter is either very restricted or does not occur. In the great majority of instances the inoculum was overgrown by *Trichoderma viride*, which suggests that the failure of *F. annosus* to grow may be associated with a type of antagonism. *F. annosus* in fact shows little or no capacity to invade litter in the plantations, for the fungus was never isolated from small branches, twigs, or needles which had been embedded in sporophores for some time. This debris normally contains species of *Penicillium*, *Trichoderma viride*, and other fungi.

F. annosus often grew better on unsterilized soil from a depth of 8 or 15–30 cm. than on humus or litter. The acid soils had a smaller proportion of samples supporting a moderate growth of *F. annosus* (groups 3–5 mm. and $>$ 6 mm.) than had the alkaline ones, but the distinction between the two types of soil was not marked in this particular test. The growth of *F. annosus* on unsterilized soils has not been recorded before, possibly because weaker inocula and an insufficient variety of soil samples were used. In this experiment, however, growth on unsterilized soil was never profuse. Since much of the work carried out on root-disease fungi has shown that these must have a substantial 'base' before infection takes place (Garrett, 1944), it seems unlikely that *F. annosus* could ever achieve infection from a limited growth in the soil.

Field observations are of interest in this connexion. Mycelium of *F. annosus* was never seen growing into the soil even from heavily infected roots. The white pustules often present on the surface of such roots contained viable mycelium only when they were freshly formed; older pustules were discoloured yellow and contained other fungi. This suggests that other fungi prevent *F. annosus* from growing into soil. Another significant fact is that *F. annosus* was found in the root systems of only a small proportion (less than 4 per cent.) of pines killed by suppression. If the fungus were widely present in the soil or litter, it might be expected to invade a greater proportion of such trees, whose resistance just before death is presumably low. Infection experiments, to be described in a later paper, show that *F. annosus* does not necessarily enter even these few weakened trees from the soil, for such trees can be infected through stem wounds, which are often present, by means of airborne spores.

During this investigation *F. annosus* was never found on the root systems of trees planted on former arable or heath sites unless stumps were present in the immediate vicinity. The examination of root systems of pines blown over by a severe gale in March 1947 showed this relationship clearly. In two areas where thinning had previously been carried out, 16 out of 23 such trees had *F. annosus*, mainly in the lateral roots. In comparable sites only a short distance away, where there had been no previous thinning, the fungus was not found in the roots of 30 blown trees. Since roots are known to die during the normal growth of pines (Büsgen and Münch, 1929), opportunities for colonization by a fungus such as *F. annosus* would be likely to occur if it grew freely in the soil. The above evidence suggests strongly that mycelial growth does not occur widely in the soil, but is limited to root systems in certain situations. This does not rule out the possibility that spores of *F. annosus* are washed into the soil and remain viable for a short period, as is suggested by experiments to be described in a later paper. It is probably true to say, however, that *F. annosus* is not present to an extent capable of causing infection in plantations on former heath or arable land until stumps are produced by thinning or other operations.

In another experiment suspensions of basidiospores, obtained by washing off fresh spore deposits on glass slides with sterile distilled water, were sprayed on to the surface of moist soil samples in Petri dishes, which were then incubated at 22·5° C. for 10 days. Mycelium of *F. annosus* never appeared unless the soil was previously sterilized by autoclaving. It appears unlikely, therefore, that the fungus can colonize soil under natural conditions by means of basidiospores.

The ability of *F. annosus* to grow on pine roots was also tested. Roots were washed to remove soil, but the bark-scales were left intact as far as possible. Two 5-cm. lengths were placed parallel and a small distance apart in a sterile Petri dish moist chamber, and a pine-root inoculum of the type already described laid on them across the centre at right angles. Dishes were incubated in containers for 14 days at 22·5° C., and the surface growth of mycelium from the inoculum recorded. With root lengths previously sterilized by autoclaving, growth from the inoculum was always abundant. With similar unsterilized root lengths, growth was variable and was classified in groups as follows: 0, none; 1, slight; 2, moderate; 3, abundant. In one experiment roots taken from a depth of 20 cm. in the soil of two plantations, one of which was formerly chalk grassland (site A) and the other *Calluna* heath (site B), were tested in this manner. Since earlier experiments had suggested that the surface growth of *F. annosus* on roots from site B was likely to be poor, a test was made at the same time with roots from site A which had previously been encased by bark-scales taken from roots of site B. The results obtained from these three series of root samples, each ten in number, are illustrated in Pl. XII, B, and recorded in Table II.

TABLE II

The Surface Growth of F. annosus *on Unsterilized Pine Roots*

Type of root sample.	No. of samples giving a growth from the inoculum in the groups:			
	0.	1.	2.	3.
From former chalk grassland (site A)	0	0	0	10
„ „ *Calluna* heath (site B)	5	3	2	0
Composite: site A roots encased by site B bark-scales	2	6	2	0

There is a striking difference in the growth of *F. annosus* on roots from sites A and B. Since it can be shown that the growth rate of the fungus in the wood of such root samples is unrelated to the type of site from which the roots are taken, it is probable that purely surface differences were responsible for the result. This is supported by the fact that the failure of *F. annosus* to grow out from the inoculum on to roots of site B was often associated with overgrowth by *Trichoderma viride*. Furthermore, growth of *F. annosus* on roots from site A plus bark-scales from site B was almost as restricted as it was on intact roots from site B, which strongly suggests that the bark-scales of roots from this site contained fungi antagonistic to *F. annosus*.

These findings correspond closely to the state of affairs under natural conditions: a superficial mycelium of *F. annosus* is often present on diseased pine roots in the alkaline soils of former arable and chalk grassland, but very seldom on similar roots in acid soils of former woodland or *Calluna* heath. The ability of *F. annosus* to grow relatively freely on roots in alkaline soils is almost certainly associated with a deficiency of competing fungi, such as *Trichoderma viride*, on the root surface.

Basidiospores of *F. annosus* germinated and produced mycelium readily on the surface of sterilized roots. Mycelium was seldom produced on unsterilized roots under similar conditions, but the chance of establishment was increased by cutting to expose the bark and wood. Establishment of the fungus on living roots by means of basidiospores is therefore theoretically possible under natural conditions, especially where wounding occurs. Basidiospores produced mycelium readily on freshly sawn sections of pine stem, unless there was heavy contamination with other fungi. The possibility of stump infection by this means cannot therefore be disregarded.

(iii) *The interaction of* F. annosus *and* Trichoderma viride *on laboratory media*

Trichoderma viride commonly overgrows *F. annosus* on agar, soil, and wood in the laboratory at 22·5° C. In addition, it often replaces *F. annosus* in stumps under natural conditions. For these reasons, and because *T. viride* was already known to affect the parasitism of *Rhizoctonia solani* and other fungi, the interaction of the fungus with *F. annosus* was studied. Ten representative strains of *T. viride*, isolated from East Anglian soils or from pine roots, were used for this work. Observations were also made on two strains, producing gliotoxin and viridin respectively, kindly supplied by Dr. P. W. Brian.

In one experiment the interaction on malt agar at 22·5° C. was investigated. A colony of *F. annosus* was allowed to develop from an agar inoculum on one side of a Petri dish for 5 or 6 days before an inoculum of the faster growing *T. viride* was placed on the opposite side. The hyphae of *F. annosus* were always checked when they came in contact with those of *T. viride*, and some strains of the latter were similarly checked at first. However, all ten East Anglian strains of *T. viride* and the two provided by Dr. Brian eventually grew through the *F. annosus* colony, although their growth sometimes continued by submerged hyphae only. When this growth of *T. viride* was complete, attempts to reisolate *F. annosus* failed and microscopic examination showed that most if not all of the hyphae were dead. Colonies of *F. annosus* kept under similar conditions but in pure culture retained viability throughout the period of the experiment.

Although *F. annosus* grows most rapidly at about 22·5° C., it must rarely encounter temperatures as high as this under natural conditions. The average soil temperature over a period of 4 years at a station in Breckland, for instance, varied between 3·6° C. (January) and 16·9° C. (August) at a depth of 30 cm. It was thought important, therefore, to discover whether the interaction with *T. viride* was similar at lower temperatures. As a preliminary, their relative growth rates on malt agar were measured. The ten strains of *T. viride* already mentioned and ten of *F. annosus*, all but one from East Anglia, were used for this comparison. Growth rates were measured for two Petri-dish cultures of each strain at 10, 5, and 0° C., each colony being measured along two diameters at right angles. The figures in Table III are the average colony increases in radius per day in mm.

TABLE III

Growth rates of F. annosus *and* T. viride *on malt agar*

	10° C.			5° C.			0° C.		
	Max.	Min.	Av.	Max.	Min.	Av.	Max.	Min.	Av.
F. annosus	2·7	2·1	2·5	1·7	1·1	1·4	0·6	0·3	0·4
T. viride	6·4	1·7	4·5	3·7	0·3	2·0	0·1	0·0	0·0

At 10° C. *T. viride* grew faster than *F. annosus*, at 5° C. the two fungi grew at approximately the same rate, while at 0° C. *T. viride* hardly grew at all. Three strains of *T. viride* giving rapid overgrowth of *F. annosus* at 22·5° C. were then tested for their interaction with this fungus at lower temperatures. The malt-agar cultures were incubated at 22·5° C. until the fungus colonies had almost met, and were then transferred to 10, 5, and 0° C. The results are given in Table IV.

TABLE IV

The Interaction of F. annosus *and* T. viride *on malt agar*

+ denotes overgrowth by *T. viride*.
— denotes no overgrowth by *T. viride*.

Temp. (° C.).	Incubation period (months).	Strains of *T. viride*		
		1.	2.	3.
10	3	+	+	+
5	4	+		—
0	6	—	—	—

In one instance at 5° C. and in one at 0° C. *F. annosus* had started to overgrow *T. viride* very slowly. There is thus a tendency for the action to be reversed at low temperatures. This result has a bearing on some of the earlier observations. It is interesting, for instance, to find that the records of free *F. annosus* mycelium beneath sporophores were all obtained in late October or November, when the air temperature was below 10° C. The growth rate of *F. annosus* on unsterilized natural substrates is so slow below 10° C., however, that extensive colonization of soil or litter is unlikely to occur under these conditions.

It was possible to study the action of *T. viride* more closely by growing the two fungi on a thin malt-agar film mounted on a coverslip in a van Tieghem cell. Under these conditions some strains of *T. viride* had an effect on the *F. annosus* hyphae well before contact occurred. The *F. annosus* hyphae were wider than usual and produced numerous short branches almost at right angles; later they became extensively vacuolated and often burst, liberating a yellowish globule of disorganized protoplasm (Pl. XII, C and D). The effect was similar to that described by Weindling (1932) for *Rhizoctonia solani* but, although the *T. viride* hyphae lay very close to those of *F. annosus*, they did not enter them.

In view of recent work by Brian and Hemming (1945) and by Brian et al. (1946) on the production of antibiotics by *T. viride*, the activity of sterile extracts prepared from the ten strains was tested. The strains were grown in Weindling's medium (adjusted to pH 3·5) for 4 days at 25° C., and the cultures were then Seitz-filtered. The method of assay closely followed that described by Brian and Hemming (1945), except that conidia of *F. annosus* were used instead of those of *Botrytis allii*. Successive twofold dilutions of the extract to be tested were made with half-strength Weindling medium. Spore suspensions were added and mixed, and three drops were withdrawn from each on to a sterile microscope slide. The slides were placed in a moist atmosphere and incubated at 22·5° C. for 48 hours, when they were examined by microscope. The activity of the extract was measured by the highest dilution inhibiting germination of conidia to less than 1 per cent. The results are given in Table V.

TABLE V

The Activity of Seitz-filtered Extracts of T. viride *Strains, as shown by their Effect on the Germination of* F. annosus *Conidia*

Maximum dilution of extract inhibiting germination	No. of strains of *T. viride*.
0 (i.e. no dilution)	7 (East Anglian)
$\frac{1}{4}$	2 (,,)
$\frac{1}{2}$	1 (,,)
$\frac{1}{64}$	2 (Dr. Brian)

Germination was not inhibited in the controls.

When dilutions of gliotoxin were tested with separate suspensions of conidia and basidiospores of *F. annosus*, germination was inhibited up to the same dilution with each. Since Brian and Hemming (1945), working with *Botrytis allii*, record an inhibition of germination at far higher dilutions than those given above for conidia of *F. annosus*, the latter are probably less sensitive to toxins of *T. viride* than conidia of *B. allii*. Nevertheless, a marked difference in activity between the East Anglian strains of *T. viride* and those from Dr. Brian is evident. With none of these strains did a relation appear to exist between the ability to overgrow *F. annosus* on malt agar and the production of toxin, as judged by this method. *T. viride* can undoubtedly kill aerial hyphae of *F. annosus*, as happens on the glass surface above agar cultures in Petri dishes, and it is therefore likely that, when hyphae of the two fungi are in intimate contact, the local production of toxin is greater than the tests indicate.

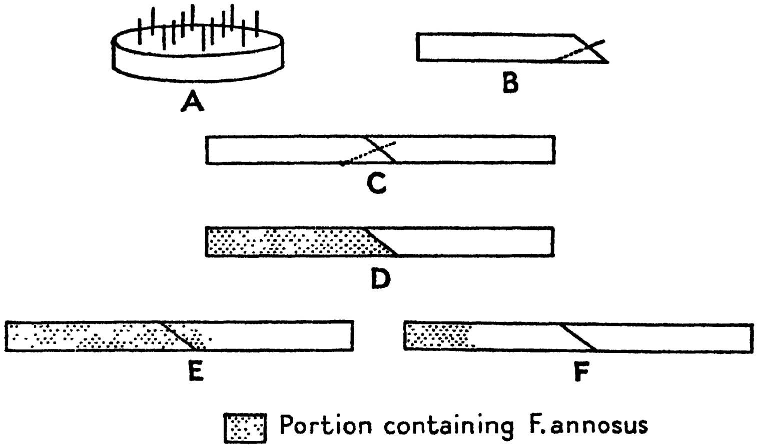
It may be concluded from these experiments that many strains of *T. viride* are antagonistic to *F. annosus*, although the effect is less marked below 10° C.

(iv) *The interaction of* F. annosus *with other fungi in pine roots*

Since there is extensive competition between fungi in stumps and dead roots, it is interesting to investigate the interaction of *F. annosus* with other fungi in woody tissues. A technique was therefore evolved by which fungi could be grown in pine roots. In order to obtain reproducible results it was necessary to obtain uniform growth of the fungi throughout the root samples and to use the roots soon after such growth was complete. For this, wood inocula were preferred to agar cultures. Inocula were prepared from blocks of pine wood about 5 cm. in diameter and 0·7 cm. thick. Twelve small pins were pushed through each block, which was then moistened and sterilized by autoclaving. The pine blocks (Text-fig. 1, A) were transferred to agar cultures of the fungi and incubated at 22·5° C. until they were well permeated by mycelium.

Meanwhile, freshly collected pine roots of diameter 0·5–1·0 cm. were cut into 5-cm. lengths, washed, and stripped of loose superficial bark. Each length was cut squarely at one end and obliquely at the other. A pin was fixed in each so that it projected through the obliquely cut end (Text-fig. 1, B). No pins, however, were inserted into root lengths to be inoculated with *F. annosus*. The roots were sterilized by exposure to the vapour of propylene oxide in stoppered bottles for 3 days, the same period being allowed for the dispersal of the vapour through the slightly raised stoppers. The root lengths were then firmly attached to the pine block inocula by fixing the squarely cut ends on to the projecting pins. Each inoculum, with twelve lengths of pine root attached, was incubated at 22·5° C. in a moist, sterile, wide-necked bottle. The root lengths were ready to use shortly after hyphae appeared on the obliquely cut ends.

In order to study interaction, root lengths containing *F. annosus*, as described above, were attached to roots containing other fungi so that the obliquely cut surfaces were adjacent (Text-fig. 1, C and D). Each pair, kept in position by the pin fixed earlier, was placed in a moist sterile test-tube. These tubes were incubated in covered containers either at 5° C. or at 15° C. For comparison, pairs were also made up in which root lengths artificially infected by *F. annosus* were replaced by lengths of naturally infected, rotted root. After a suitable interval (see Table VI) the root lengths were removed, split longitudinally, and reincubated for a short time until the relative positions of *F. annosus* and the other fungi in the wood could be observed. To prevent any great change in the relative positions of the fungi, this reincubation was carried out at 5° C. Advance (+) or replacement (—) of *F. annosus* was graded 1, 2, or 3; 3 indicating that either process was nearly complete. 0 indicated that there was no advance or replacement. Two possible results of interaction are illustrated in Text-fig. 1, E and F. The results of interaction between *F. annosus* and some other fungi are given in Table VI. Each figure of advance or replacement is an average obtained by assessing the interaction in three pairs of root lengths incubated under the given conditions. The fungi were isolated from pine roots or stumps in East Anglian plantations, with the exception of *Trichoderma viride*, strain 5, which was isolated from Breckland soil, and *Ceratostomella pini*, which was obtained from the Forest Products Research Laboratory, Princes Risborough. The basidiomycetes *Peniophora gigantea* and *Stereum sanguinolentum* were particularly abundant in pine stumps.



TEXT-FIG. 1. Diagrams illustrating a technique for studying interaction in pine roots (× ½). A, wood block of the type used as inoculum; B, root length with pin inserted; C, a pair of root lengths in position; D, the position of *F. annosus* before incubation; E, one possible result of interaction, scoring +1; F, another possible result, scoring −2.

In artificially infected root lengths at 5° C., *F. annosus* was replaced to some extent by two strains of *Trichoderma viride*, by *Torula ligniperda*, and *Peniophora gigantea*. The replacement of *T. viride*, strains 1 and 5, by *F. annosus* at this temperature parallels the reversal of interaction already described for agar cultures (Table IV). At 15° C. all four strains of *T. viride* replaced *F. annosus* rapidly, as did *Torula ligniperda* and *Peniophora gigantea*. On the other hand, *F. annosus* replaced the two species of *Ceratostomella* to some extent at both temperatures. 'Blue-stain' fungi similar to these sometimes invade pine roots soon after felling, and the result just given agrees with the observation that *F. annosus* often replaces them as it grows out from stumps

TABLE VI

The Interaction, as measured by Advance (+) *or Replacement* (—), *of* F. annosus *with Other Fungi in Lengths of Pine Root*

	Root lengths containing *F. annosus*			
	Artificially infected Incubated		Naturally rotted Incubated	
Root lengths containing the fungi.	at 5° C. for 6 weeks.	at 15° C. for 2 weeks.	at 5° C. for 6 weeks.	at 15° C. for 2 weeks.
Trichoderma viride Pers. ex Fr., str. 1 .	+1	−3	−2	−3
" " " " 2 .	−1	−3	−3	−3
" " " " 4 .	−2	−3	−3	−3
" " " " 5 .	+2	−2	−3	−3
Torula ligniperda (Willkom) Sacc. .	−2	−3	−3	−3
Ceratostomella pini Münch . .	+3	+2	0	0
C. pilifera (Fr.) Winter . . .	+1	+1	−2	−3
Peniophora gigantea (Fr.) Massee .	−1	−2	−3	−3
Polystictus abietinus (Dicks.) Fr. . .	+1	+1	−2	−2
Stereum sanguinolentum (A. & S.) Fr. .	0	0	−2	−2

along the lateral roots. Stages in the replacement of *F. annosus* by *Peniophora gigantea* in stumps have often been seen. The results obtained for the other basidiomycetes suggest that there is little or no interaction with *F. annosus*, a conclusion which is supported by the observation that the parasite often exists in stumps with these fungi for a considerable period. In the naturally rotted root lengths *F. annosus* was presumably at some disadvantage with the other fungi, which could draw on the more substantial food-base of the fresh, artificially infected lengths. However, the very free growth of these other fungi into the rotted lengths suggests that *F. annosus* is readily replaced in rotted wood by many other fungi. The extent of replacement did not differ appreciably during the respective incubation periods at 5° C. and 15° C.

That *F. annosus* is often unable to compete with other fungi in dead roots is suggested by an experiment in which root lengths artificially infected with *F. annosus* were paired in the manner already described with samples of dead roots taken from pines killed by suppression. A great variety of fungi, including basidiomycetes, are found in such roots. For comparison, similar root lengths containing *F. annosus* were paired with root samples taken from living pines adjacent to the dead suppressed ones. After 2 weeks at 15° C. the extent to which *F. annosus* had advanced in these root samples was determined and graded as before. The results are given in Table VII. There was thus little or no growth in root samples from the dead suppressed trees but vigorous growth in those from the living trees.

The status of *F. annosus* is now clearer. It characteristically inhabits stumps and roots, in which it may remain for many years actively rotting the wood, and seldom if ever grows in soil or decomposing litter. By means of spores or mycelium it can colonize freshly cut pieces of root or stem provided that these

TABLE VII

The Growth of F. annosus *in Root Samples obtained from Dead (Suppressed) and Living Pines*

Root samples.		No. giving growth of *F. annosus* in the groups.			
Origin.	No.	0.	1.	2.	3.
Dead trees	18	13	5	0	0
Living trees	11	0	0	1	10

are not too heavily contaminated by other fungi. *F. annosus* is readily replaced in wood by various strains of *Trichoderma viride* and by *Peniophora gigantea.* Although it can invade wood already occupied by certain other fungi, its powers of invasion are limited; in this respect it resembles *Armillaria mellea* (Leach, 1939). For this reason it is probable that *F. annosus* infects trees through dead roots only rarely, if at all. This is contrary to the opinion of some earlier workers such as Lagerberg (1936), who were concerned with butt rot. In East Anglia pine roots appear to be normally healthy before invasion by *F. annosus*, a condition also found in similar outbreaks of disease in California (Wagener and Cave, 1946). In the plantations investigated stumps are the primary sources of infection, *F. annosus* passing from stump roots into living roots which touch them. As has been stated elsewhere (Rishbeth, 1949), stumps are infected in the first instance by air-borne spores of *F. annosus.* The observations and experiments which demonstrated this will be described more fully in a forthcoming paper.

SUMMARY

Fomes annosus has recently caused serious damage in some East Anglian plantations of *Pinus sylvestris* and *P. nigra* var. *calabrica.*

A summary of existing information about *F. annosus* is given. Most previous investigations on the parasitic activities of the fungus have been concerned with butt rot; in the present work, however, attention has been directed to the type of root disease associated with the killing of pines.

The areas affected have a low rainfall and sandy soils, which show considerable variation chemically. Trees are often killed on former woodland sites soon after replanting, but serious disease appears on former arable or heath sites only after thinning of the plantations. Affected trees regularly have *F. annosus* in some part of the root system.

The fungus is present in stumps in every site where the disease has developed. Infection takes place by the contact of living roots with ones already infected. The first trees to be killed are infected from stump roots, but such trees may transmit the disease to adjacent ones. This leads to the formation of considerable gaps, which are often circular.

Field observations suggest that *F. annosus* does not grow freely in the soil, but is confined to woody tissues. Growth of *F. annosus* on samples of

unsterilized litter or humus was either very restricted or did not occur. Limited growth was obtained on soil samples from depths of 8 and 15–30 cm. The fungus grew well on the surface of unsterilized pine roots taken from a plantation on former chalk grassland, but poorly on roots taken from a plantation on former *Calluna* heath. This difference was probably due to the abundance of competing fungi, such as *Trichoderma viride*, in the latter site.

An account is given of the interaction in culture of *F. annosus* with strains of *T. viride*, many of which are antagonistic to the former.

A technique is described by which the interaction of fungi in roots was studied. Although *F. annosus* can invade roots already occupied by certain fungi, notably some causing 'blue-stain', its powers of invasion are limited. It is rapidly replaced in roots and stumps by *Trichoderma viride* and *Peniophora gigantea.*

The writer is indebted to Professor F. T. Brooks, F.R.S., who gave valuable help and encouragement during the investigation, and Dr. S. D. Garrett for discussion and suggestions during its later stages. Thanks are due to many officers of the Forestry Commission for their assistance. Grateful acknowledgement is also made to the following: the Director of the Forest Products Research Laboratory, Princes Risborough, for identifying cultures of basidiomycetes and for supplying some cultures of 'blue-stain' fungi; Mr. E. W. Mason, of the Commonwealth Mycological Institute, for identifying certain fungus cultures; Dr. P. W. Brian for providing two strains of *Trichoderma viride*; and Messrs. F. T. N. Elborn and P. M. B. Walker for taking the photographs.

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EXPLANATION OF PLATES

PLATE XII

A. A pine root recently invaded by *F. annosus*, showing white mycelium where the superficial bark has been removed (↓) and resin exudation (right), which has caused soil to adhere. (× ¼.)

B. The growth of *F. annosus* on unsterilized pine roots. In some instances white mycelium of *F. annosus* can be seen growing out from the inoculum, which is laid over two roots. (× ¼.) Upper row: root samples from plantation on former chalk grassland (A), supporting good superficial growth of *F. annosus*. Middle row: root samples from plantation on former *Calluna* heath (B), supporting poor growth. Spore clusters of *Trichoderma viride* can be seen on the filter paper. Lower row: root samples from site A encased with bark scales from those of site B, also supporting poor growth.

C. Photomicrograph of normal hyphae of *F. annosus* growing in a thin film of malt agar. (× 200.)

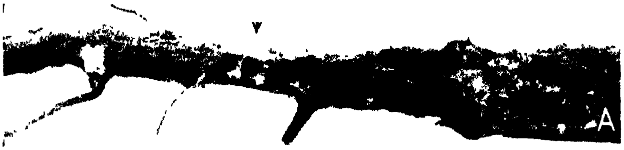
D. Photomicrograph of similar hyphae affected by toxin diffusing from a colony of *Trichoderma viride* about 2 mm. away. (× 200.)

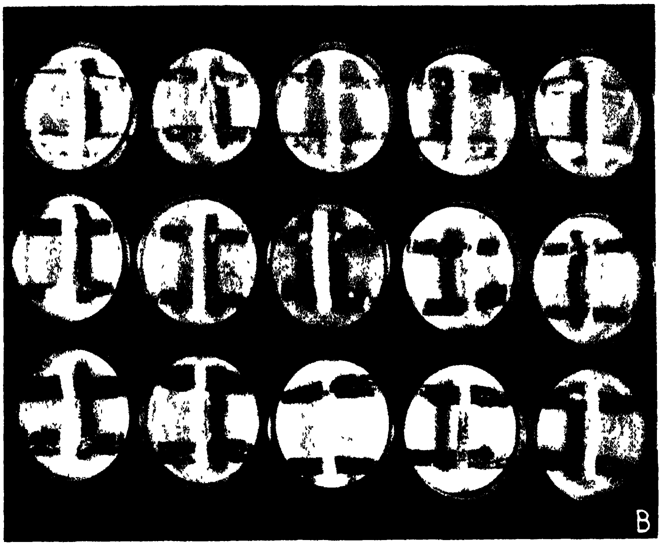
PLATE XIII

A. Photomicrograph of conidial heads of *F. annosus* formed during the incubation of a piece of infected root in the laboratory; side view. (× 80.)

B. Photomicrograph of conidial heads of *F. annosus* produced under conditions similar to the last; surface view, showing their characteristically dense formation. (× 60.)

C. A small Scots pine (*Pinus sylvestris* L.) killed by *F. annosus*, showing its roots in contact with infected stump roots (↓).





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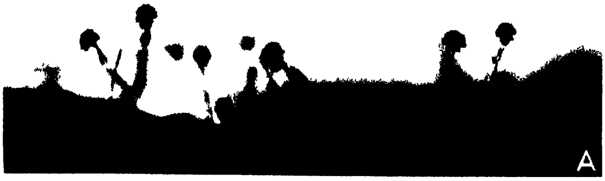

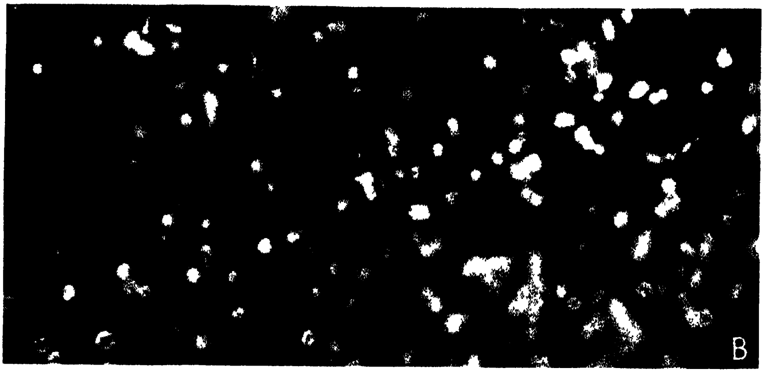

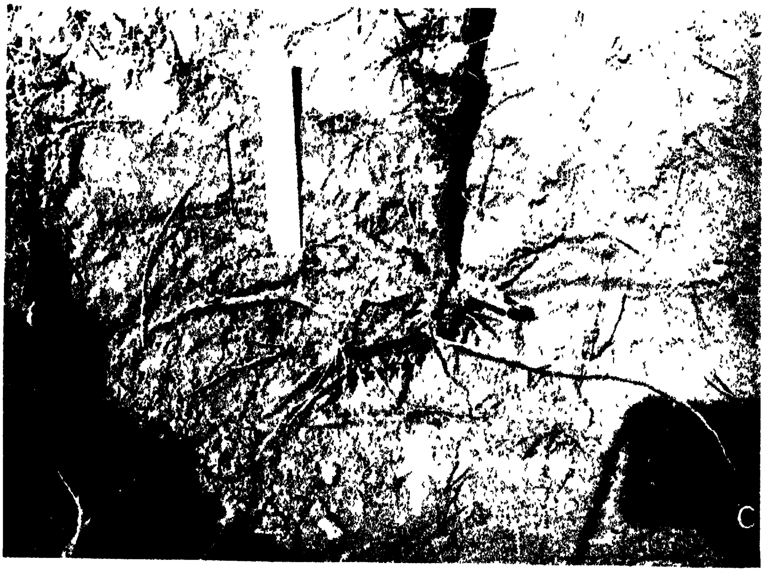

J. RISHBETH

The Relation between Cell Dimensions, Osmotic Pressure and Turgor Pressure

BY

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With one Figure in the Text

IT appears always to have been assumed from the earliest expositions of Ursprung and Blum (1916–24) and Thoday (1918) onwards that on the intake of water by a plant cell the turgor pressure increases in linear relation to the cell volume. Diagrams after Thoday (1918) based on this assumption are repeatedly reproduced: the idea is implicit in many accepted calculations. Relative volumes of cells in different conditions of turgor have moreover been estimated by cubing the ratios of over-all cell lengths or diameters and such estimated volumes have been assumed to be indicative of the relative turgor pressures and inversely of the osmotic pressures in the different states. Inasmuch as calculations and arguments are frequently based upon these assumptions, Lyon (1942), for instance, having based upon them an argument to prove the existence of a non-osmotic force in the water relations of potatoes, it appeared the suppositions were worthy of closer scrutiny. On examination they both turn out to be incorrect. This results from failure to recognize two important principles, which, if taken into account, profoundly modify the conclusions to be reached from arguments in which calculated turgor and osmotic pressures are involved. The calculated plasmolytic-cryoscopic discrepancies of Lyon (1942), for instance, readily fall to less than half their values when the principles to be put forward below are taken into account and may even disappear altogether. The data therefore take on a very different complexion when presented as evidence for a non-osmotic force in the case under investigation.

The principles which have escaped attention are (*a*) that the osmotic pressure should (neglecting small corrections for changes in degree of dissociation, hydration, imbibition, &c.) be taken as inversely proportional not to the volume of the whole cell or block of tissue (as by Lyon, 1942) but to the volume of the vacuole only, and (*b*) that there can be no linear relation between the turgor pressure and any cell dimension or the cell area or volume: its relation to the linear dimensions must, if the cell-wall material obeys Hooke's Law, be hyperbolic. Some cells, though we do not yet know how general this phenomenon may be, evidently have anomalous elastic properties and do not obey Hooke's Law (cf. data by Stow, &c., quoted by Tamiya, 1938, and Thoday, 1949), the modulus increasing with the extension and the stretching therefore

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being relatively greater at first and falling off. In such cases a linear relation is conceivable as a resultant of two superimposed effects but remains unlikely. The principle to be discussed may operate together with other principles and its effect may or may not be offset by them, but that it must always operate is mechanically inevitable.

The terms concerned will be used as follows:

Suction pressure = diffusion-pressure deficit (Meyer, 1938; Currier, 1944) = water-absorbing power (Thoday, 1918) = 'Suction force' = *Saugkraft* (Ursprung, 1916, &c.) = osmotic pressure of bathing solution with which the cell would be in equilibrium on the assumption that only purely osmotic forces are concerned = osmotic pressure—turgor pressure.

Turgor pressure = outwardly directed pressure on the cell wall (tending to burst the cell) in dynes per sq. cm. of area of inner surface of cell wall on which it acts.

Wall pressure = the inwardly directed reaction to turgor pressure = the pressure in dynes per sq. cm. of area of the protoplast, exerted on the cell contents by reason of the tension in the stretched wall. Wall pressure is (*at equilibrium*) necessarily equal and opposite to turgor pressure.

All the above terms are as used and defined by Molz (1926).

Wall tension = force per unit transverse sectional area of cell wall in dynes per sq. cm. of wall material. It results from stretching of the wall by turgor pressure and is decided in magnitude by the amount of stretching and the modulus of elasticity of the wall material. Young's Modulus for the wall substance would be given by

$$Y = \frac{\text{wall tension}}{\text{relative extension}},$$

whence wall tension $= Y \times \frac{l}{L}$, l being the increment in length of an original length L, and l/L the relative extension of the wall. It may be noted that l/L is equal to the relative change in any linear dimension of the cell.

In pursuance of criticism (*a*) above, it has to be remembered that not all the constituent phases of a block of tissue or even of a single cell are susceptible to changes in volume in response to changes in the osmotic properties of the bathing solution. The vacuole is the part of the cell most generally recognized as undergoing such volume changes. On the view that the inner protoplasmic membranes are principally responsible for osmotic swelling and plasmolytic phenomena (cf. Höffler, 1940), the vacuole might indeed be the only constituent of the cell to exhibit osmotically induced volume changes, and in this case the whole of the change in volume of a block would be due to that of the vacuoles only. It is true that if the outer membranes also possess any appreciable degree of semi-permeability to the solution concerned, the protoplast will also be expected to contribute some measure of osmotic volume changes and that it might in any case undergo some change in volume through changes in imbibition. Imbibitional changes can, however, only be relatively small and osmotic volume changes of the protoplast, if the latter be supposed to maintain a volume in equilibrium with the osmotic concentrations of the vacuole and bathing solution, can only be brought about in so far as the outer membrane is semipermeable, and its degree of functional semipermeability (cf. Höffler, 1940) is probably relatively slight. The chances are then that the protoplast, which may represent as much as 30 per cent. of the volume of the cell (Currier, 1944), will not participate markedly in volume changes. In any case, whatever the position with respect to the protoplast (which like the other points at issue is amenable to experimental investigation shortly to be undertaken), it is certain that there are other constituents of a block of tissue which cannot actively participate in volume changes. The cell walls, the intercellular spaces, and all casual constituents of the cells such as oil drops or starch grains must all be reckoned as almost completely static in this respect. In calculating the osmotic pressure of cell sap in any one osmotic state from the osmotic pressure in another state and a volume ratio, it is obviously the ratio for the vacuoles only which should be taken into account and not the volume ratio for the whole cell or block of tissue in the different states.

The extent of osmotic dilution of the cell sap by absorbed water, always assumed in the literature to be related to cell volume by a straight line, thus depends upon the change in volume of the water phase of the vacuole alone and does not therefore necessarily bear any simple relation to the volume of the whole cell or whole block of tissue. The aggregate volume of all the 'static' constituents not contributing to volume changes should be deducted from the total volume of tissue and only the remainder taken to represent the extensible vacuole: changes in volume should be reckoned on this remainder and not on the total volume. On this basis a given change in volume will of course appear as a larger percentage change in vacuole volume, and a change in osmotic pressure may appear considerably larger than when calculated on the basis of the volume changes of the whole cell or block. Where the vacuoles are relatively small or there is a large volume of cell inclusions such as starch, the percentage changes may easily appear at least three times as great on this basis. Since it is argued that the vacuoles may normally be the only important contributors to osmotically induced changes in volume, it is evident that errors arising from basing calculations on the volume changes of whole blocks of tissue or even of single cells may be very considerable.

Criticism (*b*) is less simple. It is generally realized in connexions of diverse kinds from problems in surface tension and the design of boilers to the strain on heart muscle with increased ventricular volume that the larger a container becomes the greater is the stress necessary in its walls to resist a given internal pressure, and the greater is the strain produced in the walls by any given pressure. Conversely, the smaller the container the greater is the pressure set up inside by a given strain in the walls. The application of this well-known principle to cells expanded by turgor pressure, however, appears to have completely escaped attention. That it cannot be justifiably ignored on the grounds of the small range of variation of cell size in most cases can be immediately shown by quantitative calculations as well as by the simple consideration that many cells expand by as much as 30 per cent. or more of their flaccid dimensions, and that the range of turgor pressures involved is from zero to the maximum decided by the osmotic pressure of the cell contents. In these circumstances it cannot be assumed that the results on the assumption of an arithmetic relationship will be practically the same as those on a hyperbolic basis. It is also incorrect to suggest that any reasonably satisfactory approximation may be achieved by taking the turgor pressure as proportional to the length or area of the cell instead of the volume, since, as will be shown, it may become more nearly proportional to all these functions in turn. The best approximate law therefore depends upon the range of values as well as on the Young's Modulus for the wall substance, so that the most suitable approximation to apply cannot be predicted.

The true relation between turgor pressure and cell dimensions for a cell with an isotropic wall obeying Hooke's Law will be realized from the following considerations.

Consider a partially turgid spherical cell.

Let R = the mean radius (i.e. the radius measured to the centre of the cell wall = mean of inside and outside radii) at incipient plasmolysis,
r = the increment in mean radius due to turgor pressure, and
d = the thickness of the cell wall in flaccid condition.

Then the cross-sectional area of wall material at incipient plasmolysis

$$= 2\pi Rd,$$

the mean area of cell wall (= mean of inside and outside surface areas)

$$= 4\pi R^2,$$

and the volume of wall material = mean area × thickness

$$= 4\pi R^2 d.$$

When a cell stretches, the mean area of wall increases in proportion to $(R+r)^2$. If the volume of wall material be assumed to remain constant and stretching only to alter its shape,

$$4\pi R^2 d = \text{const.} = k \text{ (say)}.$$

Hence d varies inversely as $(R+r)^2$ or

$$d = k/(R+r)^2.$$

The cross-sectional area of wall in any state

$$= 2\pi(R+r)d = 2\pi(R+r)k/(R+r)^2 = 2\pi k/(R+r).$$

Let F = the total force between the two imaginary hemispheres of the cell, due to turgor pressure and equal and opposite to that due to wall pressure, let W = the wall tension, or force per unit area of cross-section of wall, and let Y = Young's Modulus for the wall material.

Then $$Y = W \times \frac{R}{r} = \frac{F}{\text{wall area}} \times \frac{R}{r},$$

or $$Y = \frac{F}{2\pi k/(R+r)} \times \frac{R}{r} = \frac{FR(R+r)}{2\pi kr}$$

and $$F = 2\pi krY/R(R+r).$$

But Turgor pressure, T, = force per unit area on median plane of cell

$$= F/\pi\left(R+r-\frac{d}{2}\right)^2,$$

or, neglecting the half wall-thickness as small compared with R,

$$\begin{aligned} T &= F/\pi(R+r)^2 \\ &= \frac{2\pi krY}{\pi R(R+r)^3} = \frac{2krY}{R(R+r)^3} \\ &= K'r/(R+r)^3, \quad \text{where } K' = 2kY/R \\ &= K'r/(R^3+3R^2r+3Rr^2+r^3), \end{aligned}$$

or, neglecting powers of r which are small compared with R,

$$T = K'r/R^2(R+3r) = Kr/(R+3r),$$

where $K = K'/R^2$.

Hence Turgor pressure $= K\dfrac{r}{R+3r}$.

A plot from this equation gives a rectangular hyperbola with an intercept R (the radius at incipient plasmolysis) at $T = 0$. The form of the graph is shown by the heavy curve in Fig. 1.

The steepness of the curve obviously depends partly upon K, which

$$\begin{aligned} &= K'/R^2 = 2kY/R^3, && \text{since } K' = 2kY/R \text{ (above)} \\ &= 2d(R+r)^2Y/R^3, && \text{since } k = d(R+r)^2. \end{aligned}$$

The turgor pressure, therefore, though in any case bearing a hyperbolic relation to a linear dimension of the cell, increases more rapidly with linear extension the thicker the cell walls (d), the greater the elasticity modulus (Y), and the smaller the initial size of the cell.

Conversely, considering extension as a result of turgor pressure, it is evident that the linear dimensions of a cell, always bearing a hyperbolic relation to the turgor pressure, increase the more rapidly with turgor the thinner the cell walls, the smaller the elasticity modulus, and the larger the cell.

The relation may be made clearer by substituting simple hypothetical values in the equation, letting K and the initial radius = unity, and plotting the turgor pressure and relative cell radii, areas, and volumes for extensions of 10, 20, 30, &c., up to 100 per cent. The values obtained from the equation $T = r/(1+3r)$ are given in Table I and plotted in Fig. 1. The range plotted

TABLE I

r.	$R+r$.	W ($\propto r$).	Area $\propto(R+r)^2$.	Volume $\propto(R+r)^3$.	$R+3r$.	Turgor pressure (relative values $= 100r/(R+3r)$).
0·0	1·0	0	1·0	1·0	1·0	0·0
0·1	1·1	1	1·21	1·32	1·3	7·7
0·2	1·2	2	1·44	1·73	1·6	12·5
0·3	1·3	3	1·69	2·20	1·9	15·8
0·4	1·4	4	1·96	2·75	2·2	18·2
0·5	1·5	5	2·25	3·38	2·5	20·0
0·6	1·6	6	2·56	4·1	2·8	21·41
0·7	1·7	7	2·89	4·92	3·1	22·6
0·8	1·8	8	3·25	5·84	3·4	23·52
0·9	1·9	9	3·7	6·7	3·7	24·4
1·0	2·0	10	4·0	8·0	4·0	25·0

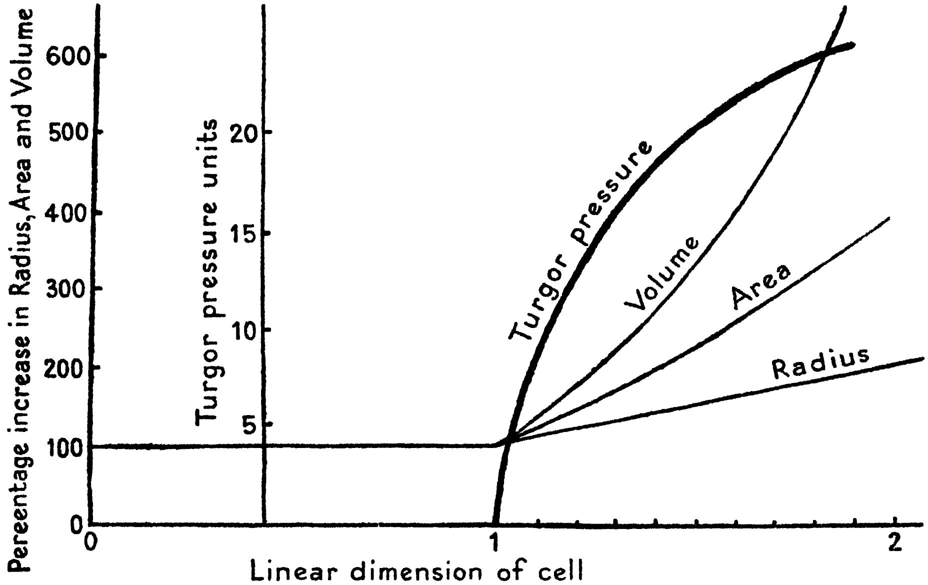

FIG. 1. Plots of the hypothetical figures given in Table I. A linear dimension, e.g. the diameter, of a cell is supposed to be unity in the condition of incipient plasmolysis. The thin curves represent respectively the percentage increases in radius, area, and volume as the cell expands, all plotted on the same scale. The thick curve represents the corresponding increase in turgor pressure in the arbitrary units of Table I, and is a plot of the equation $T = KR/(R+3r)$, being therefore a rectangular hyperbola. For further explanation see text.

(up to 100 per cent. increase in diameter) is more than that of which most cells are capable. The wider range makes the principles and differences between the graphs more obvious, but the differences over normal experimental ranges are sufficient to lead to serious errors if calculations are made on the assumption of proportionality to cell volume.

It may be seen from the graph (Fig. 1) that, although the steepness of the curves naturally depends upon the units and scales chosen, with increase of a linear dimension of the cell the corresponding turgor pressure rises most rapidly at first and later less rapidly. Of the curves to cell dimensions that for volume naturally rises most steeply, that for area less so, and that for radius least. The turgor pressure, therefore, at very small cell extensions runs most nearly parallel with the volume of the cell, at greater extensions it is most closely parallel with the area, and for greater extensions still with the radius, there being, however, no good approximation to proportionality to any of these functions. The slopes of the curves and therefore the ranges over which these generalizations apply moreover depend upon the modulus of elasticity, the thickness of the cell walls, and the actual size of the cell, so that no generalization can be safely applied. Any sort of extrapolation or interpolation may be wildly incorrect unless a full calculation be made taking the hyperbolic relation to true vacuole volume into account.

It may be noted, though it may be of less practical application than the general relation, that the full equation for the turgor pressure will be

$$T = \frac{2rd(R+r)^2Y}{R^3(R+3r)}.$$

It is now possible to examine the effect of applying these principles to some existing calculations and conclusions. Those of Lyon (1942) will serve well as an example.

Lyon (1942) determined the suction pressures and lengths of blocks of potato tuber in the flaccid, normal, and fully turgid states. From the ratios of the lengths he calculated the relative volumes by cubing. From the ratios of these volumes he obtained the theoretical values for the osmotic pressure of the original (normal) material. He then calculated the osmotic pressure at saturation by multiplying that at incipient plasmolysis by the inverted volume ratio and took this value as equal to the turgor pressure at saturation. The original turgor pressure was calculated from this by multiplying by the ratio of the difference between the volumes of normal and plasmolysed blocks to the difference between the volumes of saturated and plasmolysed blocks. (This, of course, is assuming a linear relation between turgor pressure and block volume.) The original suction pressure was then calculated from the difference between these calculated values for the original osmotic pressure and turgor pressure. Lack of agreement with the experimentally observed value of the suction pressure of the original material was taken to show the inadequacy of the purely osmotic view and that a non-osmotic force was concerned.

It is instructive to examine one of Lyon's calculations and to make a recalculation from his data, taking into account that the turgor pressure bears a hyperbolic, not linear, relation to the extension, and that the ratios of volumes of vacuoles in the different states should be used instead of the ratios of the volumes of the whole blocks.

Lyon's own data and calculation will be summarized first. Altering the format of presentation slightly to facilitate comparison with the new working they run as shown in Table II.

TABLE II

Data and Calculation after Lyon with Lay-out adapted for Comparison with Table III

		At incipient plasmolysis.	In original condition.	At saturation.
Measured suction pressures	=	21·5	8·7	0·0 atm.
Measured lengths of blocks	=	0·595	0·619	0·658 cm.
Calculated relative volumes	=	2,106	2,380	$2{,}860 \times 10^{-4}$ cm.^{3}
Osmotic pressure at incipient plasmolysis = suction pressure at incipient plasmolysis				
∴ Calculated original osmotic pressure = $21{\cdot}5 \times \frac{2{,}106}{2{,}380}$	=		19·05 atm.	
Calculated osmotic pressure at saturation = $21{\cdot}5 \times \frac{2{,}106}{2{,}860}$	=			15·88 atm.
∴ Turgor pressure at saturation (= osmotic pressure at satn.)	=			15·88 atm.
∴ Original turgor pressure $= \frac{15{\cdot}88 \times (2{,}380 - 2{,}106)}{(2{,}860 - 2{,}106)}$	=		5·77 atm.	
Calculated original suction pressure = O.P. − T.P. = 19·05 − 5·77	=		13·28 atm.	
Observed value (above)	=		8·7 atm.	
Discrepancy = 13·28 − 8·7	=		4·58 atm.	

In making a first recalculation from Lyon's data it will be assumed that the aggregate vacuole volumes in Lyon's material amounted to half the total volume of tissue. This appears to be a fair estimate for a material like potato in which such a large proportion of the cell volume is occupied by starch: it may even be too great. It is evident that determinations would have to be made of the actual proportion of the block volume which took part in osmotic volume changes. Such determinations would probably be made, for the sake of a more stable basis of reference, not upon original material in an arbitrary condition but upon saturated material mounted in water. It will therefore be assumed for the purposes of calculation that the vacuoles in Lyon's material represented one-half of the total volumes of the blocks in the saturated condition. It will further be assumed that the principles proved above for spherical cells apply closely enough to polygonal cells and therefore that the symbols R and r for the flaccid radius and increment of radius on swelling of a spherical cell may be applied equally well to any linear dimension (e.g. length) of a cell and its corresponding increment on swelling, or therefore to the length and increment in length of a whole block. The blocks will be assumed to swell to the same relative extents as individual cells. Lyon's data are recalculated on these assumptions in tabular form in Table III, the format corresponding for ease of comparison with that of Table II.

TABLE III

Recalculation from Lyon's Data

		At incipient plasmolysis.	In original condition.	At saturation.
Measured suction pressures	=	21·5	8·7	0·0 atm.
Measured lengths of blocks	=	0·595	0·619	0·658 cm.
Calculated volumes of blocks	=	2,106	2,380	$2{,}860 \times 10^{-4}$ cm.^{3}
If vacuole volumes = half total volumes at saturation, half the saturated volume (2,860/2) or 1,430 units remains constant.				
∴ Vacuole volumes (total volumes −1,430)	=	676	950	$1{,}430 \times 10^{-4}$ cm.^{3}
∴ Ratios of osmotic pressures	=	1,430 :		676
		and 950 :	676	
Osmotic pressure at saturation $= \frac{676}{1{,}430}$ of O.P. at incipient plasmolysis $= \frac{676}{1{,}430} \times$ suction pressure at incipient plasmolysis $= \frac{676}{1{,}430} \times 21\cdot5$ atm.	=			10·12 atm.
∴ Turgor pressure at saturation (= O.P.)	=			10·12 atm.
Ratios of linear dimensions (from measured lengths)	=	100 :	104 :	110·5
Whence increments of linear dimensions, r	=	0	4	10·5
∴ Turgor pressures $= \frac{Kr}{R+3r}$	=	0	$\frac{4K}{112}$	$\frac{10\cdot5K}{131\cdot5}$
∴ $\frac{10\cdot5K}{131\cdot5} = 10\cdot12$, whence $K = 126\cdot8$				
∴ Original turgor pressure $= \frac{4 \times 126\cdot8}{112}$	=		4·54 atm.	
The original osmotic pressure $= \frac{676}{950}$ of the osmotic pressure at incipient plasmolysis $= \frac{676}{950} \times 21\cdot5$	=		15·3 atm.	
Original suction pressure = O.P. − T.P. = 15·3 − 4·54	=		10·76 atm.	
Observed value (from above)	=		8·7 atm.	
∴ Discrepancy = 10·76 − 8·7	=		2·06 atm.	

The recalculation shows that when the true relation between turgor pressure and extension and an estimate of the relative vacuole volume are taken into account, the discrepancy of 4·58 atm. obtained by Lyon and taken as evidence for a non-osmotic force may fall to less than half its value. This is reproduced as the first value obtained by this method of recalculation founded on the first guess at the relative vacuole volume. The relative vacuole volume obviously needs estimating, but meanwhile it may be noted that further calculations show that if the relative vacuole volume be taken as 40 per cent. the discrepancy becomes only 0·66 atm., and if taken as about 38 per cent. there is no discrepancy at all, the calculated agreeing with the observed value. At a relative vacuole volume of 33 per cent. the discrepancy becomes 1·7 atm. the other way on, the observed value being the greater. Any of these relative vacuole volumes is equally possible. It may be noted that the elasticity modulus need not be known, but that unless the relative vacuole volume is known and taken into account the data mean nothing. Although the writer is in no way anxious to disprove the existence of non-osmotic forces in cell water relations, it must be agreed that these findings considerably alter the complexion of Lyon's work when presented as evidence for the action of non-osmotic forces in the water relations of potato tissue. It is hoped to repeat this type of experiment side by side with determinations of relative vacuole volumes.

I am very grateful to Professor W. H. Pearsall and Professor D. Thoday for helpful comments.

SUMMARY

It is shown that the relation between cell extension and turgor pressure for isotropic cells obeying Hooke's Law is not linear but hyperbolic. There can be no approximate linear relationship to any cell dimensions satisfactory for purposes of calculating turgor or osmotic pressures.

In calculating osmotic pressures of saps at different cell extensions it is important to use the ratios of volumes of the vacuoles only and not those of whole cells or blocks of tissue. This applies to all calculations of natural osmotic pressures from observed osmotic values.

Recalculations from existing data show the profound effect of taking these principles into account. In particular the data of Lyon are shown to provide no evidence for a non-osmotic force in potato tubers.

Determinations of the relative vacuole volumes should always be made and used when osmotic pressures are calculated from volume ratios. The hyperbolic relation of turgor pressure to the linear dimensions of the cells must also be taken into account.

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Studies in the Genus *Fucus*

II. The Anatomical Structure and Chemical Composition of Receptacles of *Fucus vesiculosus* from Three Contrasting Habitats[1]

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With four Figures in the Text

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INTRODUCTION

SEASONAL variations occur in the chemical composition of many brown seaweeds (Lapicque, 1919; Lunde, 1940; Dillon, 1943; Black, 1948), but no attempt has been made to correlate these variations with seasonal activities such as periods of active growth and reproduction. In *Fucus vesiculosus* the fertile regions of the thallus are sharply defined and form swollen receptacles at the apices of lateral branches. Their development proceeds over a period of several months, and collections of these fertile tips have been made at different developmental stages, to study the changes both in anatomical structure and in chemical composition which take place during receptacle development. Sterile tips were collected from the same plants at the same time as fertile tips,

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[1] This investigation formed part of a thesis accepted for the Degree of Doctor of Philosophy in the University of London, 1948.

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so that any seasonal variations in the chemical constituents distinct from those connected with fruiting would become apparent.

Vegetative plants of *Fucus vesiculosus* collected from contrasting habitats have been shown to exhibit differences in both anatomical development and chemical composition (Moss, 1948). The influence of environment is again considered in relation to anatomical development and chemical composition of receptacles from the same three habitats. These were selected on account of their varying degrees of exposure to wave action, which may result in further differences such as variations in mineral supply.

Although early stages in the development of the conceptacles of *Fucus vesiculosus* have been studied (Bower, 1880; Roe, 1916; Nienburg, 1913), the structure and development of the receptacle as a whole has received little attention.

DEVELOPMENT AND ANATOMICAL STRUCTURE OF A RECEPTACLE

External morphology

The receptacles of *Fucus vesiculosus* are borne at the apices of lateral branches, which have dichotomized at frequent intervals compared with the longer internodes of the vegetative leaders (Knight, 1947). Externally the young receptacle is first apparent as a slight prolongation of the midrib from the thallus apex, with no development of the wings. Even in the mature receptacle the distinction between midrib and wings, which is so marked a feature of the vegetative thallus of *Fucus vesiculosus*, is not apparent.

Apical growth

During the very early stages of receptacle development a large apical cell lies at the base of the apical groove, similar to that first described by Woodworth (1888) at the vegetative apex. As the receptacle enlarges and becomes 3–4 mm. in length, the apical groove flattens out, and in the half-grown receptacle it is replaced by a convex tip.

During flattening out of the apical groove the identity of the single large apical cell becomes obscured. The convex tip of a mature receptacle is occupied by small compact cells surrounded by meristoderm. After the apical cell ceases to function, diffuse growth takes place throughout the receptacular tissues, accompanied by the deposition of mucilage. This diffuse growth in the fertile tips is maintained only during gamete maturation, for as soon as the gametes have been liberated, necrosis of the receptacular tissues sets in.

The meristoderm

The meristoderm is a tissue of maximum importance in receptacle development, for it is from this layer that ultimately the conceptacles are derived (Nienburg, 1913). In the young receptacle the cells of the meristoderm are brick-shaped with their longitudinal walls adjacent. Rapid surface growth accompanies enlargement of the receptacle, and the cells of the meristoderm undergo repeated anticlinal divisions. When seen in surface view they appear associated in groups of two to eight cells, representing products of recent segmentations. In the very young receptacle periclinal divisions also take place in the meristoderm and the segments cut off towards the interior develop into the cortex of the mature receptacle (Fig. 1). By the time of gamete extrusion the meristoderm has lost its meristematic activity and the cells are isodiametric. This loss of meristematic activity may be associated with the decay of receptacles after gamete extrusion.

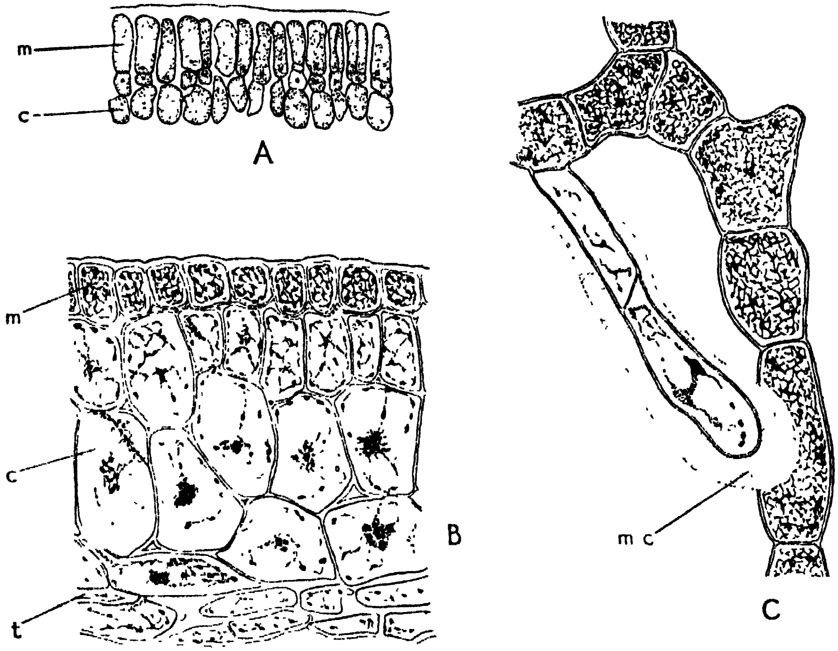

FIG. 1. A and B. Development of cortex in receptacles of *Fucus vesiculosus*. A in young receptacle, B in mature receptacle.

× approx. 500.
c = cortex.
m = meristoderm
t = layer of thick-walled cells.

C. Branching of medullary filament in receptacle of *Fucus vesiculosus*. × approx. 500.
m.c. = mucilage sheath.

The cortex

During secondary differentiation of the vegetative thallus of *Fucus vesiculosus*, secondary cortex originates as a result of both radial and tangential divisions in the cells of the primary cortex. But in the mature receptacle no secondary cortex is formed. As active surface growth of the receptacle is taking place, the original cortical initials laid down near the apex enlarge and become pigmented.

Behind the vegetative apex cortex grades into the central medulla, but in the receptacle there is a layer of thick-walled cells sharply separating these two tissues. These thick-walled cells are similar to those figured and described as 'mechanischen Systems' in *Himanthalia lorea* (Wille, 1910). Close to the apex cell-division in this layer is irregular, and a small-celled tissue with few intercellular spaces is laid down. The thickening of the cell-walls is uneven, and gives an immediate blue reaction with iodine and concentrated sulphuric acid, indicating the presence of cellulose. To keep pace with the rapid surface growth during receptacle enlargement, these thick-walled cells get stretched, especially in the longitudinal direction (Fig. 1, C). Although they are differentiated early in receptacle development, this layer of thick-walled cells is not continued into the thallus immediately beneath the receptacles.

The central medulla

In the very young receptacle, where the apical groove is still present, there is equal deposition of mucilage with separation of the filaments throughout the medulla. Progressive increase in the deposition of mucilage takes place from the outer medulla towards the centre, until the centre of a mature receptacle is occupied by mucilage traversed by a network of medullary cells. These remain in contact through simple pit connexions, similar to those developed in the vegetative part of the thallus.

Even in young receptacles the medullary cells branch freely in all directions, while elsewhere in the vegetative thallus branching of medullary cells is rare. The branches arise as lateral outgrowths from the original medullary cells, and are often protected in a thick envelope of mucilage (Fig. 1, C). Within the receptacle the medullary cell-walls remain thin, whereas in the older parts of the vegetative thallus they become extremely thick.

Secondary hyphae

In the vegetative apices hyphae have not been found closer to the apex than 4 mm., whereas in the mature receptacles they are present at the extreme tip. They originate even from the small cells at the extreme tip, and grow horizontally before they eventually turn down into the tissues of the receptacle. Elsewhere in the mature receptacle hyphae run in all directions through the intercellular mucilage, in contrast with the sterile tips where hyphae are directed toward the attaching disc. At the base of mature receptacles hyphae, originating from the sterile tissues below, grow up into the fertile region. In old receptacles, after gamete extrusion, hyphae have also been seen growing out from cells comprising the conceptacle wall. Secondary hyphae probably develop in the receptacles of many species of the Fucales, but their presence seems to have been noted hitherto only by Wille (1910) in *Himanthalia lorea* and Laing (1941) in *Bifurcaria laevigata*.

COMPARISON OF RECEPTACLES FROM THREE CONTRASTING HABITATS

Receptacle development proceeds over a period of several months. From each of the three localities, Cullipool (with strong exposure to wave action), Loch Melfort (sheltered), and Aird's Point (moderate exposure), receptacles were at similar developmental stages when collections were made during the same month.

In December the young receptacles which were collected for chemical analysis were up to 8 mm. in length and 3 mm. in breadth at the base, tapering to a convex tip. At this early stage they were surrounded by a very active meristoderm. As little intercellular mucilage had developed, a central strand of medullary cells was still present and few secondary hyphae had developed. In these young receptacles in December there was a gradation in conceptacle development from the base to the apex, the oldest conceptacles being differentiated at the base. These already contained numerous antheridia or oogonia. In the latter two to eight nuclei were visible, although none showed cleavage into distinct ova. At the same time, the conceptacles at the tips of these receptacles were very small and neither oogonial nor antheridial initials were visible.

Mature receptacles were collected from each locality in June, those from Cullipool (exposed habitat) being much larger than receptacles from either Loch Melfort or Aird's Point. In spite of differences in size both the anatomical structure and developmental stage of antheridia and oogonia were similar from the contrasting habitats. In these mature receptacles the conceptacles were full of ripe gametes from the base to the extreme tip. Firm mucilage traversed by a network of medullary cells filled the centres of these receptacles, and secondary hyphae meandered in all directions.

Two months later, in August, receptacles from all localities had shed most of their gametes. The conceptacles contained numerous exo-chitons, and sometimes a few young oogonia or antheridia along the base of the conceptacle wall. At this late stage most of the intercellular mucilage had been lost with gamete extrusion, and many of the receptacles had been damaged, probably by waves or browsing animals.

In vegetative parts of well-grown thalli it has been observed that cortical and hyphal development is most marked in plants from the open sea (Moss, 1948). The mature receptacles, however, of plants from the open sea were about twice the size of those from the other two habitats but were remarkedly similar as to development of cortex and hyphae. But the receptacles are only temporary structures, disintegrating as soon as the gametes have been discharged, and this may account for the apparent lack of formative response to contrasting environmental factors.

CHEMICAL COMPOSITION OF RECEPTACLES FROM THE SAME THREE HABITATS

Collection of material

The first collections were made between June 3 and June 13, 1947, when receptacles were mature. A second series of collections was made between July 30 and August 3, 1947, i.e. approximately 7–8 weeks later, when most of the gametes had been shed and the receptacles were beginning to disintegrate.

Between December 5 and 12, 1947, a third series of collections was made, when new receptacles were at a very early stage of development. This latter series will be referred to as stage I in relation to receptacle development, while June samples represent stage II and August samples stage III in this developmental series. These numbers are used in the tables of analytical results.

At each collection approximately 100 lb. of fruiting plants were gathered from each of the three localities. *Fucus vesiculosus* is dioecious and after the random collection, male and female plants were separated. Samples were then cut from each thallus as shown in Fig. 2; samples from each sex being kept separate. Samples C were taken only in June when the receptacles were mature. The small weight of December samples necessitated the mixing of both male and female receptacles from Aird's Point, and of sterile tips of male and female thalli from both Aird's Point and Cullipool. As the samples were cut off individually by hand, any tips with epithytic plant growths or attached Polyzoans were discarded. Also any receptacles on a thallus which were obviously much younger or older than the average selected for that particular sample were not included.

The samples were dried on racks above a coke furnace at the Oban out-station of the Scottish Seaweed Research Association, and afterwards ground to a fine powder in a Christy and Norris No. 8 laboratory mill fitted with a $\frac{1}{64}$-inch perforated plate screen. It was calculated that in June approximately 7,650 receptacles or 2,500 sterile tips were included in each sample, while in December some 11,000 receptacles were collected from each locality. The analytical methods used throughout are those developed by the Scottish Seaweed Research Association (Black, 1948; Cameron, Ross, and Percival, 1948).

Data obtained from fertile plants collected at Aird's Point on June 3, 1947, showed that there was an average number of 47 sterile tips to 125 fertile tips per thallus. The average fresh weight of a thallus was 87·5 g., of which the receptacles alone accounted for 49 g., i.e. more than half the total fresh weight of a fertile plant. This is of technical interest, for if harvesting of the weed for industrial purposes occurs during the reproductive period, then a greater weight of unwanted water will be included in the freshly gathered plants.

Percentage dry weight

It is difficult to obtain an accurate estimation of the percentage dry weight of parts of a thallus of *Fucus vesiculosus*. Being an intertidal species, the plants are naturally collected at low tide. At times of bright sun or drying wind their tips are often dry and crisp, and the older parts will also have lost a considerable amount of water through exposure to the atmosphere (cf. Pringsheim, 1923; Isaac, 1933; Zaneveld, 1937). In order to compensate for this loss, the material to be dried was previously soaked for 30 minutes in sea-water from the same locality as the plants.

In Table I the results obtained from samples collected at Aird's Point (moderate exposure) are summarized. Material from both Loch Melfort (sheltered shore) and Cullipool (open sea) gave similar values.[1]

TABLE I

Dry Weight (expressed as Percentage Fresh Weight)

		Stage I, Dec.	Stage II, June.	Stage III, Aug.
Male	Receptacles	16·9	11·2	9·9
	Below receptacles	—	—	—
	Sterile tips	20·5	24·9	22·0
Female	Receptacles	16·9	11·8	8·8
	Below receptacles	—	27·3	—
	Sterile tips	20·5	24·6	26·9

From plants collected at the same time, the percentage dry weights of sterile tips from both male and female plants are similar. There is also a seasonal variation in the dry weights of sterile tips. In the observations recorded higher values were obtained in August than in June, and December, or September samples (Moss, 1948). Walker (1948) has shown that whole thalli of *Fucus vesiculosus* give quite different results, the percentage dry weights being low during the summer months. A probable explanation of this is put forward later.

Receptacles of both sexes at similar developmental stages have similar dry weights. Even at a very early developmental stage the water content of receptacles is higher than in sterile tips of the same thalli graph I, Fig. 3. As the intercellular mucilage increases with maturation of the gametes, so does the water content increase. After the gametes are shed, most of the intercellular mucilage has been lost too, but the interior of the receptacles becomes filled with water so that low percentage dry weights were still obtained for receptacles collected in August.

The increase in the uptake of water accompanying maturation of the reproductive organs is localized in the receptacle itself, for when a low value of 12·3 per cent. is given for Loch Melfort male receptacles in June, the part of the thallus immediately beneath the receptacles gives a value of 26·3 per cent.; some 3 per cent. higher than the value for sterile tips from the same plants.

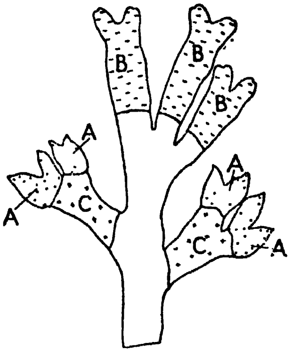

FIG. 2. Diagram showing samples of *F. vesiculosus* collected for chemical analysis.

A = Receptacles.
B — Sterile tips (approximately 1–1½ inches long).
C = Thallus below receptacles (approximately 1–1½ inches long).

The results summarized in Table I are interesting to compare with the seasonal variation in the percentage dry weights of *whole thalli* of *Fucus*

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[1] Complete tables of results of chemical analyses of material from both Loch Melfort and Cullipool are tabulated in thesis for Ph.D. degree (London) 1948.

vesiculosus, recently published by Walker (1947). His plants were collected each month from Aird's Point near Port Appin, i.e. from the same locality as some gathered for the present investigation. He states that the percentage dry weight of 'Each species (i.e. *Ascophyllum nodosum*, *Fucus serratus*, *Fucus vesiculosus*, and *Fucus spiralis*) rises and falls twice each year. It will be recalled that the diatom cycle rises and falls twice each year'. The rise and fall twice each year is not so marked in *Fucus vesiculosus* as in *Fucus spiralis*.

It was in June and August 1947 that fertile thalli of *Fucus vesiculosus* were collected from Aird's Point for the present investigation, and the dry weights of the receptacles were very low, approximately 10 per cent. As previously stated, the receptacles at this season constitute over half the total fresh weight of a thallus. Hence their effect would be to lower the percentage dry weight of a complete thallus, and it is the presence of mature receptacles on the thalli which accounts for the low values reported by Walker for July 1945 and 1946. For a previous investigation (Moss, 1948) *Fucus vesiculosus* had also been collected from Aird's Point in September 1946. At this season no fertile plants were found. Hence if thalli are devoid of fertile tips in autumn, it follows that percentage dry weights of complete thalli would be higher than when they are bearing swollen receptacles with high water content. It is at the non-fruiting period that Walker reports the highest percentage dry weights for 1946. Thus the seasonal variation in dry weights of complete thalli of *Fucus vesiculosus* from Aird's Point described by Walker can be related to the reproductive cycle of that species. The relationship to the diatom cycle is obscure.

Walker's figures for September and October are similar to those recorded for the same months for material collected at Cove and Stonehaven by Hendrick (1898), 32·09 per cent. and 35·05 per cent. respectively. Butler (1931) gives the dry weight of *Fucus vesiculosus* collected from St. Andrews, New Brunswick, in June and July as 23·6 per cent. Unfortunately the period of reproduction in this locality is not given, but this value is much higher than that obtained by Walker for plants collected off the west coast of Argyllshire during the same months.

It has been shown that a decrease in the water content of the tissues occurs from the apex to the base of a vegetative thallus of *Fucus vesiculosus* (Moss, 1948). But nowhere in the sterile thallus was there such a great difference in the water content as has now been shown to exist between the tissues of mature receptacles and sterile tips from the same plants. Moreover, at the same time water content is decreasing in certain tissues, as in the sterile tips during summer, in other parts of the thallus it may simultaneously be increasing, as during the maturation of receptacles.

Percentage total ash

The results recorded in Table II are for samples collected from Aird's Point, and very similar values were obtained for material from both Loch Melfort and Cullipool. They show extremely high values for total ash, ranging from 15·6 per cent. for Loch Melfort sterile tips in August to 40·0 per cent. for receptacles from the same plants. A survey of the literature shows that high values for the ash content of *Fucus vesiculosus* are reported elsewhere (presumably for complete thalli): 20·78 per cent. (Hendrick, 1898), 20·07 per cent. (Butler, 1931), 22·00 per cent. (Lunde, 1941).

TABLE II

Total Ash (expressed as Percentage Dry Weight)

		Stage I, Dec.	Stage II, June.	Stage III, Aug.
Male	Receptacles	25·5	35·5	37·3
	Below receptacles	—	19·5	—
	Sterile tips	23·5	18·6	17·8
Female	Receptacles	25·5	35·5	37·3
	Below receptacles	—	20·7	—
	Sterile tips	23·5	18·9	18·0

Sosa-Bourdouil (1940) found low percentages of ash in the gametes, 8·5 per cent. in the antherozoids, and 5·4 per cent. in the ova on an anhydrous basis. Thus the high ash content of mature receptacles is not associated with the gametes but with the receptacular tissues. It is interesting to note that Dawson Turner (1809) says: 'In the islands of Jura the inhabitants dry their cheeses without salt, by covering them with the ashes of this plant which abounds with such quantities of salts, that from 5 ounces of the ashes may be procured 2½ ounces of fixed alkaline salts.' The results here recorded in Table II on a dry weight basis show enormous differences between sterile and fertile tips in June and August, the fertile tips containing approximately twice the percentage of total ash as do the sterile tips from the same plants.

Lapicque (1919) found that the percentage ash of *Laminaria flexicaulis* was high in winter and low in summer. Soluble carbohydrates were low in winter when ash was high, and high in summer when ash was low. Hence he said, 'il est plausible qu'il s'agisse simplement d'une substitution isotonique'. Lapicque's calculations were on a dry-weight basis, and on a similar basis these results for *Fucus vesiculosus* show great variations during receptacle development, ash being low in December samples and high in June and August. But if calculations of the ash content are on a fresh-weight basis, then comparatively little variation takes place during receptacle development, graph II, Fig. 3. It is these calculations on a basis of fresh weight which are essential for a comparison of the chemical composition of the actual plants which are analysed.

The percentage ash on an anhydrous basis shows as marked a variation in relation to receptacle development as do any of the organic constituents. As the water content increases with maturation of the receptacles, so does the ash content in relation to the increase of other constituents.[1] At the same time the part of the thallus immediately below the mature receptacles has an ash content similar to that of the sterile tips. So that the high percentage ash on an anhydrous basis correlated with a high water content of the mature receptacles is confined to the fruiting body and does not affect the thallus beneath it, from which it differs so much in anatomical organization.

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[1] Since sending to press, it has been ascertained that this increase is mainly in water soluble constituents, especially soluble chlorides.

Percentage organic nitrogen

The total organic nitrogen was determined by the standard Kjeldahl method, and the result multiplied by 6·25 gives the percentage crude protein. In Table III are the data obtained for samples from Aird's Point. Those from Loch Melfort and Cullipool show similar variations, although the values given for Loch Melfort (sheltered habitat) are always lower than those for corresponding samples from either of the other two localities.

TABLE III

Organic Nitrogen (expressed as Percentage Dry Weight)

		Stage I, Dec.	Stage II, June.	Stage III, Aug.
Male	Receptacles	2·08	1·39	0·88
	Below receptacles	—	1·47	—
	Sterile tips	1·58	1·72	0·85
Female	Receptacles	2·08	1·05	0·63
	Below receptacles	—	1·04	—
	Sterile tips	1·58	1·70	0·93

At each developmental stage from either of the three habitats a higher percentage of organic nitrogen is present in the male than in the female receptacles. Sosa-Bourdouil (1940) found very high nitrogen content in the gametes of *Fucus vesiculosus*: 8·5 per cent. and 4·7 per cent. for antherozoids and ova respectively. The higher percentage of nitrogen in the male gametes may account for the slightly higher values always obtained for male receptacles.

Between June and August the organic nitrogen content of all samples is halved. The value of taking both sterile and fertile tips from the same plants is here very apparent, for if only fertile tips had been collected, then one might conclude that the decrease from June to August accompanied liberation of the gametes, especially as these have very high nitrogen content. But the parallel decrease in the organic nitrogen content of sterile tips shows that in these parts of the thallus there is a seasonal variation in organic nitrogen content. It is high in winter and early summer with a rapid decline in late summer, a variation distinct from and not related to fruiting. It is probably related to the supply of available nitrogen.

The great variation in organic nitrogen found between similar samples of thalli collected in June and August emphasizes the necessity of stating the time of collection of plants if analytical results are to have any scientific value. Different seasons of collecting probably account for the great differences for the organic nitrogen content of *Fucus vesiculosus* reported: 2·47 per cent. (Hendrick, 1898), 2·43 per cent. (Vincent, 1924), 2·29 per cent. (Barlow, 1911), 1·61 per cent. (Wheeler and Hartwell, 1893), 1·37 per cent. (Butler, 1931). Butler's figures are for material collected from New Brunswick in July, and as they are presumably for complete plants, they cannot be directly compared with the present results. These show that organic nitrogen of mature receptacles is low, so that during the period of fruiting the receptacles would lower the value obtained for a complete plant.

Calculations on a fresh-weight basis show that when *Fucus vesiculosus* is fruiting there is always a higher percentage of protein in the vegetative tips of thalli than in the fertile tips of the same plants, graph III, Fig. 3. The greatest difference occurs at maturation of the gametes, when there is three to four times as much protein in the sterile tips as in the receptacles. At the same season the region of thallus immediately beneath the receptacles gives a value intermediate between that of the receptacles and the sterile tips.

Percentage mannitol

The data obtained for samples collected from Aird's Point are set out in Table IV. Material from Loch Melfort and Cullipool showed similar variations in mannitol content, although the values from both the sheltered and exposed habitat were slightly higher than those from Aird's Point.

TABLE IV

Mannitol (expressed as Percentage Dry Weight)

		Stage I, Dec.	Stage II, June.	Stage III, Aug.
Male	Receptacles	8·65	5·55	5·46
	Below receptacles	—	11·93	—
	Sterile tips	10·02	12·59	15·0
Female	Receptacles	8·65	5·88	5·73
	Below receptacles	—	11·43	—
	Sterile tips	10·02	12·77	16·34

These results, whether calculations are on dry or fresh weight basis, show that there is at all stages of receptacle development a lower percentage of mannitol in the receptacles than in the vegetative tips from the same thalli, the greatest difference occurring in August when most of the gametes have been shed. While calculations on an anhydrous basis show for August samples approximately a 1 : 3 ratio of percentage mannitol in receptacles to percentage mannitol in vegetative tips, calculations on a fresh weight basis give a ratio of 1 : 4 for the same samples.

The percentage mannitol in the sterile tips increases from December to August, at the same time as it is decreasing in the receptacles (Fig. 4). This indicates that mannitol is not a storage material of either the ova or antherozoids of *Fucus vesiculosus*. Mannitol is at its maximum when organic nitrogen is at its minimum. The region of thallus immediately below the mature receptacles in June has a lower percentage mannitol than the vegetative tips, but a much higher value than receptacles from the same thalli. Thus the very low percentage of mannitol is located within the receptacle itself. The seasonal variation in the mannitol content of the vegetative apices of thalli of

Fucus vesiculosus is similar to the seasonal variations hitherto recorded for species of *Laminaria* (Lunde, 1937; Dillon, 1940; Black, 1948).

Percentage alginic acid

Table V records the alginic acid content of samples collected from Aird's Point. Similar variations were shown in material from both Loch Melfort

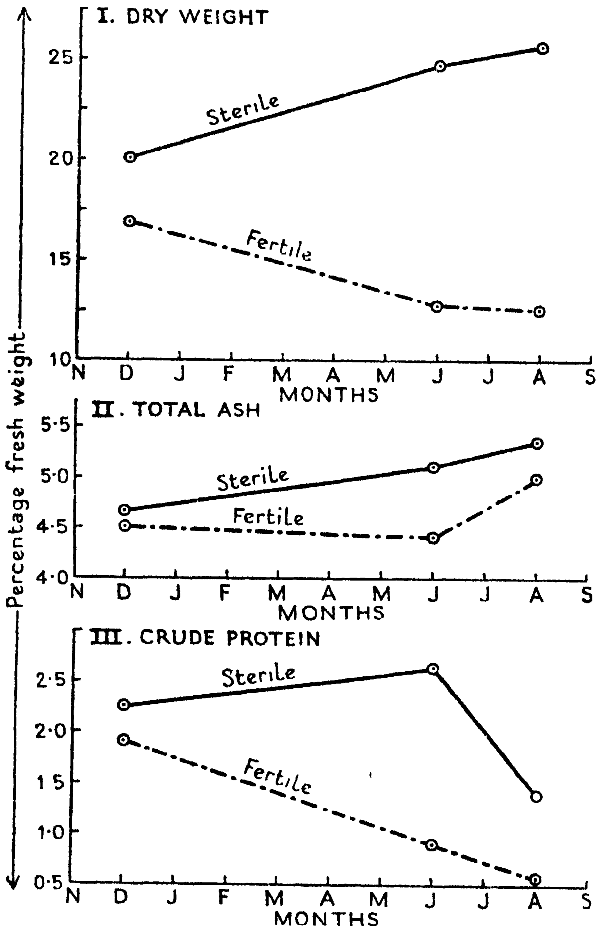

FIG. 3. Variations in dry weight, total ash and protein content (expressed as percentage *fresh weight*), of sterile and fertile tips of female thalli of *Fucus vesiculosus* from Cullipool.

and Cullipool, although sterile tips of plants from the sheltered locality (Loch Melfort) always gave lower results than those from habitats with moderate or strong exposure to wave action.

Estimations of the alginic acid content of various species have been determined, and in species of *Laminaria* it has been demonstrated that a seasonal variation in alginic acid occurs (Lunde, 1937; Dillon, 1943; Black, 1948). These investigators report that the maximum percentage of alginic

TABLE V

Alginic Acid (expressed as Percentage Dry Weight)

		Stage I, Dec.	Stage II, June.	Stage III, Aug.
Male	Receptacles	26·99	18·53	19·32
	Below receptacles	—	17·12	—
	Sterile tips	29·38	18·39	17·53
Female	Receptacles	26·99	18·01	18·22
	Below receptacles	—	16·46	—
	Sterile tips	29·38	18·28	17·42

acid in Laminaria is reached in spring. In samples of *Fucus vesiculosus* analysed a much higher percentage of alginic acid has been found in the vegetative tips of thalli in December than in either June or August. These December figures, 27·2 and 29·8 per cent. for Cullipool and Aird's Point respectively, are much higher than Lunde's figure previously given for *Fucus vesiculosus* (19 per cent.). This is presumably for complete plants, but previous results (Moss, 1948) have shown that there is a much higher percentage of alginic acid in the young vegetative tips than in other regions of the thallus of *Fucus vesiculosus.*

The greatest proportion of alginic acid is found in the sterile tips when ash and mannitol are lowest. The samples of thallus taken below the mature receptacles have a similar alginic acid content to sterile tips from the same thalli. Hence as noted previously for mannitol, the lower percentage alginic acid on fresh weight basis is confined to the fruiting body and does not affect the thallus immediately beneath it.

Fig. 4 shows the variation in alginic acid content of tips of thalli from Cullipool, calculated on fresh weight basis. In December the young receptacles have a lower percentage alginic acid than have the vegetative tips at the same season, and this decreases rapidly as the receptacles mature, and falls still farther when most of the gametes have been shed.

Unestimated residue

Although there are great variations in the chemical composition of sterile and fertile tips of the thalli collected at the same season, yet the total estimated substances in the present investigation account for similar percentages of the dried samples of both. In June and August samples 55–60 per cent. of the dried weed has been estimated, whereas the same methods account for 65 per cent. of the dried weed collected in December. This shows that in all samples there is a residue of 30–40 per cent. of the dried weed to be estimated. Preliminary analyses show that laminarin and fats constitute some of this residue.[1]

The doubling of the ash content during development of receptacles (on dry weight basis) is an interesting phenomenon, and an analysis of this ash would yield information as to the nature of this mineral accumulation associated with reproduction in *Fucus vesiculosus.*

[1] It has since been found that both laminarin and ether-soluble materials decrease during development of receptacles, so that less than 2 per cent. of each were found in mature receptacles from all habitats, on dry weight basis.

GENERAL CONSIDERATIONS

The anatomical structure and chemical composition of the receptacles of *Fucus vesiculosus* have been found to be very similar in the three contrasting habitats selected, whereas the vegetative thalli exhibited marked differences (Moss, 1948). The sterile tips which were collected for this investigation show a much higher percentage of alginic acid in plants from the open sea than from the sheltered loch.

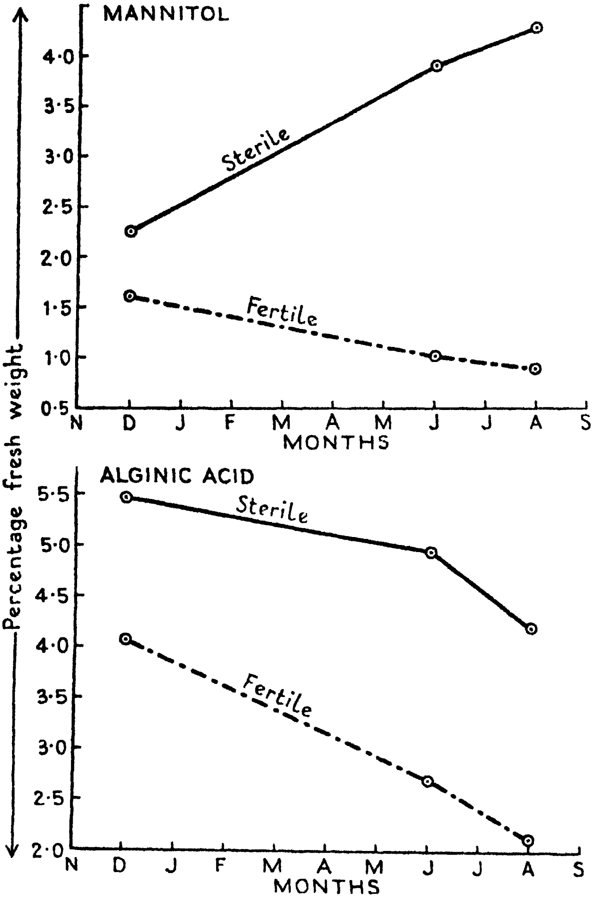

FIG. 4. Variations in mannitol and alginic acid content (expressed as percentage *fresh weight*) of sterile and fertile tips of female thalli of *Fucus vesiculosus* from Cullipool.

At the initiation of a receptacle there is a change in the metabolism of the sterile tip correlated with the anatomical changes which are also taking place.

Fucus vesiculosus is dioecious; water, ash, mannitol, and alginic acid content of plants of both sexes are similar, but organic nitrogen is slightly but consistently higher in male than in female receptacles.

Analyses of sterile tips taken from the same thalli as receptacles show that seasonal variations in chemical composition occur, differing from and even varying in the opposite direction from those connected with reproduction. Mannitol, for example, has a low value in December, reaching a maximum during late summer. In contrast, in the receptacles, mannitol is lowest in late summer. Since the receptacles may form more than half the fresh weight of a plant, this emphasizes the importance of considering the growth and reproductive cycle in connexion with variations in chemical composition of whole thalli of seaweeds throughout the year; an aspect hitherto ignored amongst the vast literature on the chemical composition of seaweeds.

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I wish to thank the Director and Staff of the Scottish Seaweed Research Association for their help and generosity in providing facilities for collecting the material used in this investigation.

SUMMARY

Stages in the development of receptacles of *Fucus vesiculosus* have been collected from three contrasting habitats off the west coast of Argyllshire, for a study of their anatomical structure and chemical composition.

No marked differences in development, anatomical structure, and chemical composition have been found in receptacles from these three contrasting habitats. They are temporary structures concerned with the period of reproduction. It is in the perennial vegetative parts of the plant that the contrasting environmental conditions of sheltered loch and open sea had more pronounced effects, both upon anatomical structure and chemical composition.

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Studies in the Genus *Fucus*

III. Structure and Development of the Attaching Discs of *Fucus vesiculosus*[1]

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With Plate XIV and three Figures in the Text

INTRODUCTION

THE basal organ in the Fucaceae consists generally of a small discoid structure adhering to the substrate, although in *Phyllospora* numerous branches like laminarian haptera arise from a central disc. *Fucus vesiculosus* occurs on a variety of substrates, and externally the attaching disc seems equally well developed whether it is on solid rock, iron girders, or wooden breakwaters. On any of these substrates it is secure enough to resist the constant strain to which the thallus is subjected by the movement of waves. Where thalli are not subjected to this constant strain, as on salt marshes, the basal organ of *Fucus vesiculosus* is not developed (Baker, 1912, 1916) and the plants are unattached. In a similar manner, the attaching discs of *Pelvetia canaliculata* (Baker, 1912*)* and *Ascophyllum nodosum* (Cotton, 1912) are absent from salt-marsh forms.

The combined features of dioecism and external fertilization have made *Fucus vesiculosus* a favourite organism for the study of polarity in relation to cleavage of the zygote (Nienburg, 1923; Whitaker, 1931, 1937, 1938). Thuret and Bornet (1878) give admirable figures of the early developmental stages of sporelings of *Fucus serratus*, *Pelvetia canaliculata*, and *Ascophyllum nodosum.* They say that *Fucus vesiculosus* resembles *Fucus serratus* in its development, but they did not trace the development of the basal organs beyond the rhizoids of the sporelings. Oltmanns' studies on fucoid sporelings (1889) and later those of Nienburg (1923) are in relation to thallus differentiation, and the classical accounts on the anatomical structure of the fucoids by Reinke (1876) and Hansteen (1892) do not trace out the developmental sequence of the attaching organs. The development of adventitious branches from the upper surface of the attaching disc is a common feature of *Fucus vesiculosus*, and their origin has been described by Oltmanns (1889).

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[1] This investigation formed part of a thesis accepted for the Degree of Doctor of Philosophy in the University of London, 1948.

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MATERIAL AND METHODS

For the early stages in the ontogeny of the attaching organs of *Fucus vesiculosus* sporelings were raised in culture, using natural sea-water, after boiling and filtering, as the culture medium. Packets of ripe oospheres and antherozoids were removed from the ostioles of discharging conceptacles with a fine brush and mixed together in a glass dish containing sea-water. Fertilized eggs sank to the bottom of the dish, and the following day early stages in sporeling development could be found. From sporelings cultured in this way development of the attaching organs could be studied for several weeks until the young sporelings became overgrown with diatoms and other micro-organisms.

Sporelings were also cultured on a substrate of pith. Discs of pith, about 3 mm. thick, were floated on a thin layer of melted paraffin wax in the bottom of a glass dish, so that on cooling the discs were firmly fixed to the dish. Care was taken to ensure that the top of the discs did not get covered with a layer of wax. Sea-water was then placed in the dish, and oospheres and antherozoids introduced and well mixed. The fertilized eggs settled on the discs of pith and there germinated.

Sporelings raised on pith remained relatively free from micro-organisms for a year, although they showed no further signs of development after 5 months when they were approximately 1·5 mm. in length. The sea-water in the culture dishes was changed about every 3 weeks, and the cessation of development might be due in part to starvation from certain nutrients. Moreover, the sporelings were maintained in culture under continuous immersion, and this may hinder the normal development of an intertidal organism. Also the culture dishes were kept near a laboratory window facing south-east, and as the culture was started in November 1946, after about 5 months' growth the young sporelings were exposed to very bright sunlight for part of each day. But these sporelings cultured on a substrate of pith remained relatively free from diatoms for a longer period than did those raised on a glass substrate. The rhizoids penetrated the pith cells and remained free of the diatoms which so often attacked the rhizoids of sporelings cultured on glass.

After 4 to 6 weeks' growth sporelings cultured on pith started to develop apical hairs, and by about 3 months each possessed an apical tuft of hairs, almost equal in length to the entire length of the rest of the sporeling. Small adventitious branches arose from the lower part of some of the sporelings after about 4 months' growth, and these also developed a tuft of apical hairs. After 6 to 8 months the apical hairs gradually fell off.

Some of these sporelings raised in culture were studied while still living, while others were fixed in formalin-acetic alcohol, sectioned and stained in gentian violet. The attachment of the sporelings to discs of pith made it easy to determine their orientation for embedding and sectioning.

For subsequent stages in the development of the attaching discs, both sporelings from the *Fucus vesiculosus* zone and adult plants were collected from a wooden breakwater at Cromer (Norfolk). Attaching discs of mature plants were also collected from rocks in a sheltered bay in Loch Melfort (Argyllshire). From south of Port Appin (Argyllshire) *Fucus vesiculosus*, growing upon *Ascophyllum nodosum* and *Fucus vesiculosus*, was obtained. These discs were fixed in formalin-acetic alcohol, and preparations were stained with gentian violet.

DEVELOPMENT OF THE ATTACHING DISC

Attachment of the sporeling

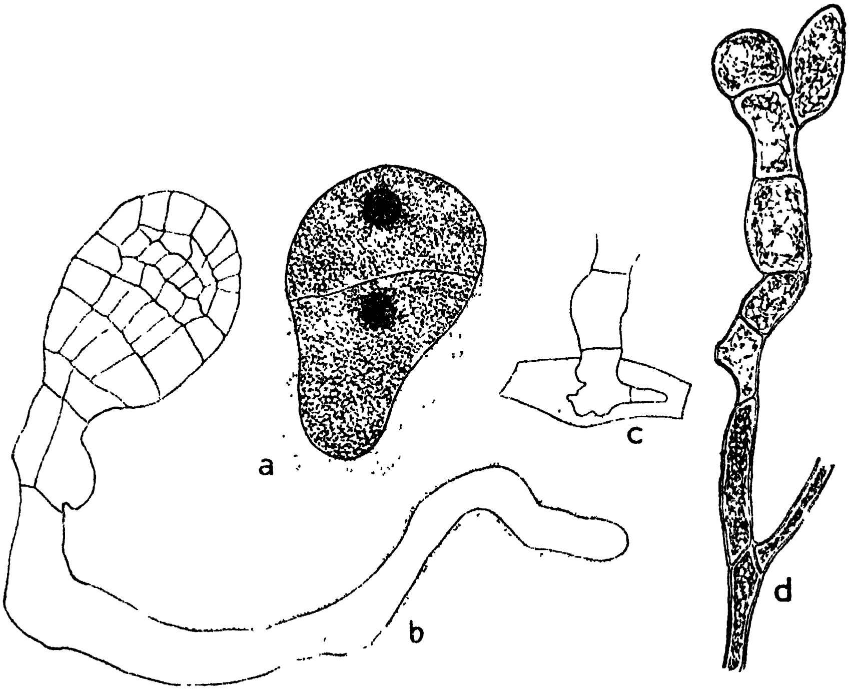
Germination of the zygote of *Fucus vesiculosus* follows soon after fertilization. Before there is any cleavage of the cytoplasm a small protuberance grows out from one side and forms the primary rhizoid. From its earliest appearance the primary rhizoid is surrounded by a thick mucilaginous envelope (Text-fig. 1 *a*). This mucilage secures the young sporeling to the substrate, for sporelings with only a slight rhizoidal protuberance surrounded by this mucilage strongly adhere to the bottom of a culture dish. This envelope of mucilage surrounding the primary rhizoid is clearly figured by Thuret and Bornet (1878) in sporelings of *Fucus serratus* and *Ascophyllum nodosum*. It is ignored in the later figures of Oltmanns (1889) and Nienburg (1923), although Oltmanns says (1922) 'Dieses besorgt alsbald die Festheftung und wird dabei unterstützt von den äussersten Membranschichten, welche stark verschleimen. Das ist auch später noch erkennbar'. The mucilage is transparent and easily overlooked under examination with bright light. However, in the early stages of germination the envelope of mucilage is very thick and is rendered more conspicuous with very dilute gentian violet. The mucilage readily takes up the stain, and if care is not exercised it becomes overstained, and the delicate membranc of the rhizoidal cell inside the mucilage layer gets obscured.

During the first 2 days of growth the primary rhizoid increases in length and a transverse wall cuts off the rhizoid from the upper cell (Text-fig. 1 *a*), which eventually gives rise to the thallus. Oltmanns (1922) figures a sporeling of this species with a much branched unicellular rhizoid, but I have never observed such branching in material cultured on a substrate of either glass or pith. The primary rhizoid increases in length until it is several times that of the rest of the sporeling. Thin, usually oblique transverse walls develop in the rhizoid so that a long filament of cells is formed, still surrounded by a thin layer of mucilage.

In the cell above the primary rhizoid, longitudinal divisions take place and protuberances from the cells thus cut off may develop into secondary rhizoids (Text-fig. 1 *b*). The tips of these rhizoids are also surrounded by a mucilaginous envelope. They undergo successive branching and many eventually become as long as the primary rhizoid, so that, in sporelings cultured on a substrate of glass, the identity of the primary rhizoid becomes obcured. When germinated on glass, the rhizoids of the sporelings are richly pigmented. After

3 to 4 weeks the mucilage envelopes of the rhizoids become heavily infested with micro-organisms.

When sporelings were germinated on discs of pith, the primary rhizoid grew down into the pith. It penetrated not only between the pith cells but also broke through the cell-walls and traversed the cell cavities. Subsequent rhizoids penetrated in a similar manner. Compared with the rhizoids growing on a glass substrate, those penetrating into pith were more richly branched. Contact with an obstacle appeared to stimulate branching, for as shown in Text-fig. 1 *c* when the apex of a rhizoid meets an obstacle (here a pith cell-wall) it swells up, becomes irregular in shape, and sends off branches in other directions where less resistance is encountered.



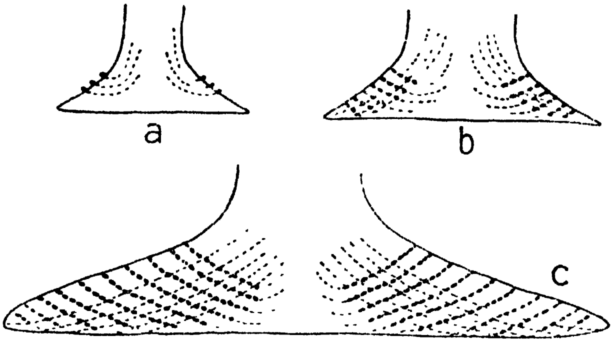
TEXT-FIG. 1. *a.* Early stage in development of sporeling of *Fucus vesiculosus*, showing mucilage sheath around the rhizoid. *b.* Development of secondary rhizoids from lower cells of sporeling. *c.* Branching at the tip of a rhizoid of a young sporeling. *d.* Upgrowing hyphal filament from the pseudo-cortex of a young disc.

With further development of the sporeling, hyphae arise from the lowermost cells of the young thallus and later from the internal tissues. Those hyphae arising from the innermost cells of the sporeling form very long narrow filaments which advance by apical growth through the tissues and eventually push their way down amongst the rhizoids. While some penetrate down into the substrate, others push their way to the surface, and spreading out over the rhizoids, begin to form a small attaching disc on the surface of the substrate.

This process is continued throughout the life of the plant. Those hyphae which penetrate to the outer limits of the disc continue to advance over the substrate and thus increase the area of the disc.

Attachment of the mature plant

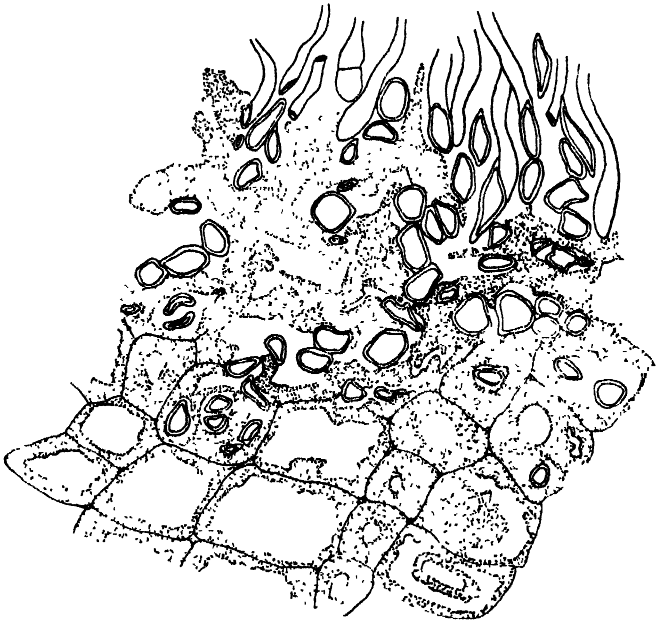
In the case of attaching organs of sporelings a few millimetres in length collected from a wooden breakwater at Cromer (Norfolk) hyphae had filled every available space in the eroded cells on the surface of the breakwater. In the disc of a sporeling about 5 mm. in length some of the hyphae coming in from the thallus interweave amongst the rhizoids and other descending hyphae, and then turn out till they reach the upper surface of the young disc. The interweaving of the hyphae in the disc resembles the warp and weft threads of a fabric, whereas in the stipe the hyphae intertwine like rope fibres.



TEXT-FIG. 2. Diagrams *a*, *b*, and *c* demonstrating development of the pseudo-cortex and parallel hyphal filaments in the disc of *Fucus vesiculosus*.

When the hyphae reach the upper surface of the disc they start to segment. Text-fig. 1 *d* shows such a hyphal filament teased from a young disc, which has produced a row of five cells at its tip. The rectangular form of these cells is in contrast with the very narrow elongated cells of the hyphae below. These cells cut off towards the exterior, having advanced beyond the mass of interweaving hyphae, enlarge, and become deeply pigmented. They are uninucleate and the outermost round off and get worn away so that the upper surface of the disc often has a layer of dead cells and debris over it. As can be seen in Text-fig. 1 *d*, the subterminal cell of an upgrowing hyphal filament frequently gives rise to a lateral branch. The close approximation of numerous of these upgrowing hyphal filaments eventually results in the formation of a 'pseudo-cortex' over the upper surface of the disc.

As soon as these upgrowing hyphal filaments have started to segment, further hyphae coming in from the thallus penetrate between them to the outer limits of the disc. The upgrowing filaments undergo further segmentation, and so continual hyphal penetration and further segmentation of the upgrowing hyphal filaments lead to the development of parallel filaments in the disc of a mature thallus (Text-fig. 2). The regularity of these filaments in the disc of a mature thallus is striking, for the cells comprising the parallel filaments are regular and rectangular in shape, and stand out in sharp contrast against the narrower and longer cells of the interweaving hyphae. Plate XIV, A, is a photomicrograph of part of the disc of a mature thallus showing the pseudo-cortex from which parallel filaments can be traced back into the disc. In a well-developed disc the hyphal filaments have cut off about a dozen cells beyond the zone of hyphal penetration and these form a pseudo-cortex over the upper surface of the mature disc. The filaments composing the pseudo-cortex may branch. Their cells are pigmented and the cell-walls remain thin, even in the parallel filaments amongst which hyphae have interwoven. In a well-developed disc these parallel hyphal filaments are absent from the immediate centre (Text-fig. 2) and this represents the position of the original attaching organ of the sporeling, before there had been any development of a pseudo-cortex. Advancement of the attaching disc over the substrate is the result of penetration of hyphae from the interior of the disc to its outer limits, so that all stages in development of the pseudo-cortex can be followed from the outer margin towards the stipe of a mature thallus.



TEXT-FIG. 3. Penetration of hyphae from the disc of *Fucus vesiculosus* into a woody substrate. × approx. 420.

The disc of a well-grown plant then, consists of a mass of interweaving hyphae covered over by the pseudo-cortex. The hyphae on the disc are generally about twice the diameter of those in the midrib of the thallus. As a result of the tortuous course which they pursue in the disc, the hyphal cells are often irregular in form and in the diameter of the cell cavity. In the disc the interweaving hyphae exhibit frequent branching, while elsewhere in the thallus the hyphae seldom branch. Branching of the hyphae in the disc is frequently followed by evection.

Text-fig. 3 shows a section taken towards the outer limit of a disc of a well-developed thallus removed from a wooden breakwater. Hyphae from the thallus have penetrated into the xylem tracheides of the wood (Pl. XIV, B) and nearest to the thallus this hyphal penetration has resulted in the breakdown of the walls of the tracheides beyond the surface layer already eroded by the sea. In the older part of the disc this has resulted in the inclusion of small pieces of lignified cell-walls amongst the hyphae.

This penetration of hyphae from the attaching disc of *Fucus vesiculosus* into a wooden breakwater is similar to the penetration observed by Grubb (1923) from the attaching organs of *Porphyra umbilicalis*. Penetration into the substrate accounts for the extreme difficulty in removing a complete attaching disc from the substrate, for the disc always brings away with it the surface layers of rock or substrate to which it is adhering. The actual penetration of hyphae into the substrate may be essential for the attachment of a thallus of *Fucus vesiculosus*, and this would account for the absence of this species from certain kinds of rock, e.g. smooth granitic rocks weathered on an exposed shore. It is true that in small cracks and fissures in the smooth surface numerous sporelings may be found, but few if any develop further.

ATTACHING DISCS FROM A SHELTERED HABITAT

While collecting plants of *Fucus vesiculosus* from a sheltered bay in Loch Melfort (Argyllshire) during September 1946 it was observed that complete attaching discs were easily removed from the substrate of granitic boulders. Numerous rudiments of adventitious branches had developed from the undersurfaces of these discs so that only small areas were adhering to the substrate. It is common to find proliferations arising from the upper surfaces of the attaching discs of this species. But in these discs there has been loss of adhesion followed by proliferation from what is normally the adhering surfaces of the discs. The occurrence of this in a sheltered habitat where there is little pulling force of the thallus to resist is interesting to compare with the complete absence of attaching discs in salt-marsh forms of *Fucus vesiculosus*.

ATTACHING DISCS FROM EPIPHYTIC PLANTS

Thalli of the Fucaceae are often covered with epiphytic growths of filamentous brown algae, but in the literature there is no reference to the epiphytic habit of *Fucus vesiculosus* itself. Yet while collecting this species off the west coast of Argyllshire, where there is such prolific growth of fucoids, plants can frequently be found growing upon *Ascophyllum nodosum* and occasionally upon *Fucus serratus* and *Fucus vesiculosus*. The thalli of both *Fucus vesiculosus* and of the host show normal development.

Sections taken through the disc of a sporeling of *Fucus* which had developed on a thallus of *Fucus vesiculosus*, about 3 in. from the apex of a vegetative branch, showed fine hyphae descending from the sporeling and penetrating well into the tissues of the host. The hyphae pushed in between the medullary cells of the host, but they did not enter any of the host cells. The cortex of the sporeling had established continuity with the cortex of the host, and the absence of any signs of cortex of the host immediately beneath the disc of the sporeling suggested that the original entry of the sporeling rhizoids was through a wound in the surface of the thallus of the host.

The photomicrographs in Pl. XIV, C and D, are of sections taken through the attaching disc of a mature thallus of *Fucus vesiculosus* which was growing upon *Ascophyllum nodosum*. The disc above the tissues of the host showed the normal structure of a disc developing upon a rock substrate, but the advancing margins of the disc had stimulated the meristoderm of *Ascophyllum* to great activity. Large and lightly staining cells were cut off by the meristoderm of *Ascophyllum* as if to form more cortex. But descending hyphae from the disc of *Fucus* penetrated between these cells, and so cortical filaments of *Ascophyllum* became included in the region of the disc. The cells of these cortical filaments grew much larger and were more rounded in form than were the original cortical cells of *Ascophyllum*. The latter provided a boundary beyond which hyphae from the disc of *Fucus* were unable to penetrate. In the centre of the disc there was close union between the cortical filaments of *Ascophyllum* and the interweaving hyphae of the disc of *Fucus*, and this association of the tissues formed a secure attachment for the thallus of *Fucus vesiculosus*. There was no penetration of hyphae into the cells of *Ascophyllum*, and no suggestion of parasitism as described by Batten (1923) for *Polysiphonia fastigiata* growing upon *Ascophyllum*. Thus the presence of these attaching discs of *Fucus vesiculosus* upon other members of the Fucaceae are examples of epiphytism, arising in a habitat where there is prolific growth of fucoids and great competition for the available substrate.

This investigation was carried out in the Department of Botany, Westfield College, during the tenure of a Postgraduate Research Studentship. I wish to express my sincere thanks to Dr. E. M. Delf for helpful criticism.

SUMMARY

1. The development of attaching organs in *Fucus vesiculosus* has been studied.

2. Attachment of the germinating zygote to the substrate is secured by a primary rhizoid, surrounded by a mucilaginous envelope. During later development attachment of the sporeling is aided by hyphae growing from the tissues of the thallus.

3. Sporelings raised on a substrate of pith show that the rhizoids and hyphae of the sporeling penetrate the interstices of the substrate.

4. Hyphae from the main thallus also penetrate into the attaching organ, push their way to the upper surface, and there segment, forming a pseudo-

cortex over the upper surface of the attaching disc, from which parallel filaments can be traced into the centre of the disc.

5. Discs collected from a wooden breakwater show numerous hyphae penetrating down into the substrate.

6. Discs of mature thalli collected from a very sheltered habitat showed the development of numerous rudimentary adventitious branches on the undersurface adjacent to the substrate.

7. Discs of *Fucus vesiculosus* growing upon thalli of *Fucus vesiculosus* and *Ascophyllum nodosum* are recorded from the west coast of Argyllshire. In the former example, hyphae penetrated between the cells of the host, while in the latter the meristoderm of the host was stimulated to great activity and the filaments of cortical cells produced became included in the disc of *Fucus*. In neither case were cells of the host entered, so that these are examples of true epiphytism of *Fucus vesiculosus*, arising in a habitat where there is great competition for the available substrate.

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DESCRIPTION OF PLATE XIV

A. Photomicrograph of part of R.L.S. through disc of mature plant of *Fucus vesiculosus*, showing parallel hyphal filaments. × 40.

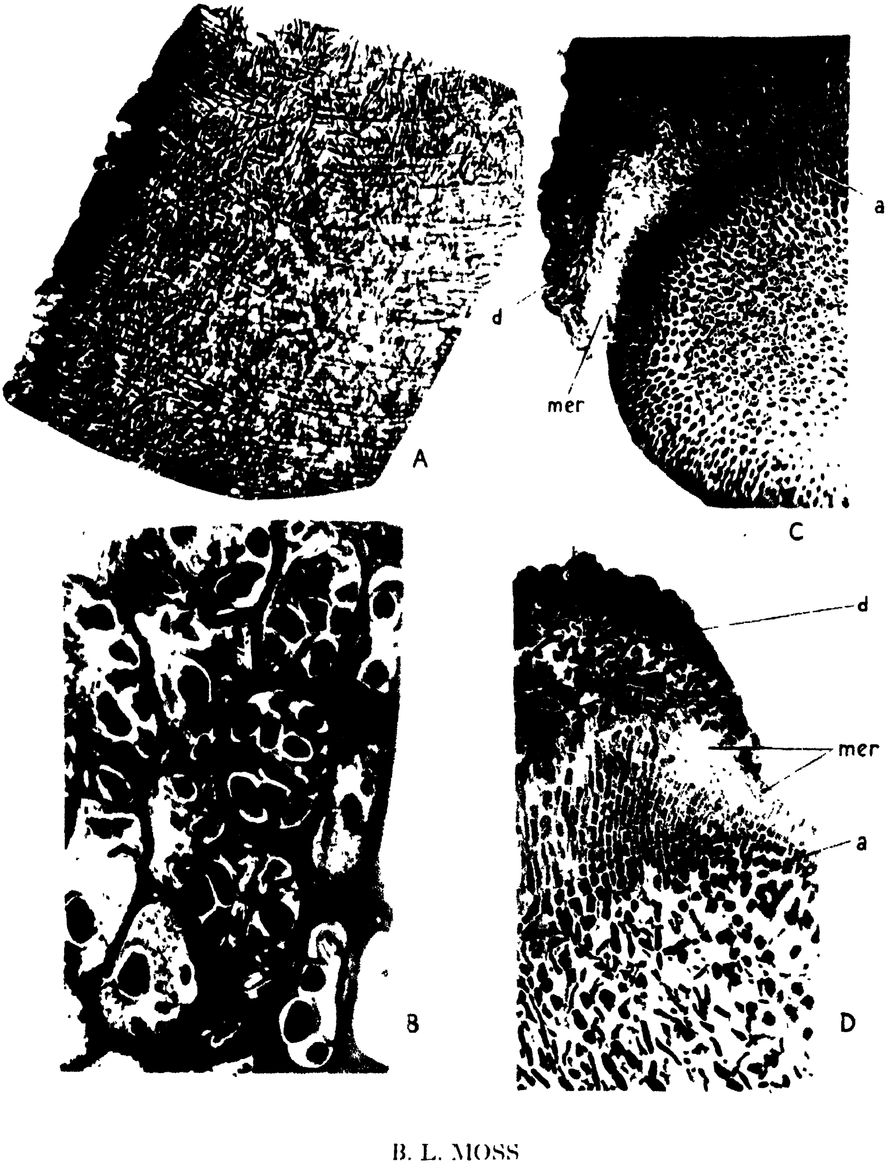
B. Photomicrograph of T.S. through a substrate of wood beneath a disc of *F. vesiculosus*, showing xylem tracheids filled with hyphae. × 380.

C and D. Photomicrographs of discs of *F. vesiculosus* epiphytic upon *Ascophyllum nodosum*, showing stimulation of the meristoderm (*mer*) to great activity.

C. Ascophyllum in transverse section. × 40.

D. Ascophyllum in longitudinal section. × 82.

d = disc of Fucus. *a* = cortex of Ascophyllum. *mer* = meristoderm.



B. L. MOSS

A Quantitative Study of Shoot Development in *Vicia faba*

I. The Xylem of the Plumule

BY

K. J. DORMER

(*University of Nottingham*)

With six Figures in the Text

ALTHOUGH the development of the vegetative shoot in dicotyledons and particularly the development of its vascular system have long been the subjects of intensive study there is still a surprising lack of quantitative data. Most of the existing morphological literature, even of the most recent, is written in general descriptive terms, and when measurements are given at all they play quite a minor role in the discussion. Apart from the biometrical work on leaf-shape recently reviewed by Ashby (1948) the intensive application of quantitative methods is found mainly in physiological studies such as the investigation by Goodall (1949) of the growth of the seedling of *Theobroma*. Understandably, though none the less regrettably, the writings of physiologists often display a combination of accurate measurement and refined statistical treatment with morphological analysis of the crudest description. This weakness is apparent both in the investigations on hormones as activators of cambial growth (e.g. Snow, 1935) and in the accounts of the anatomical changes associated with photoperiodic phenomena (e.g. Wilton, 1938; Wilton and Roberts, 1936).

The present investigation was originally undertaken in the hope of elucidating by defoliation experiments the part played by the leaf in the development of the trace bundles connected with it. Although it has been very generally assumed by anatomists that the development of trace bundles is in some way under the control of the leaf which they serve, very little experimental work has been done which can be regarded as supporting this interpretation. Not only has it not always been appreciated that the results of decapitation experiments, important though they may be in other connexions, can have little bearing on this particular problem, but most of the experiments which might have contributed to this branch of anatomy have been carried out by physiologists who have not distinguished between leaf traces and other bundles.

A study of the normal course of development of the selected material is obviously an indispensable preliminary to the actual defoliation experiments. The present paper therefore deals with the normal growth of the primary vascular system of the plumule of *Vicia faba*. Only a few of the lower internodes are described, and the account is not carried beyond the point at

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which the interfascicular cambium begins to contribute to the xylem. It is intended that later papers in this series should describe the development of axillary branches and the effects of defoliation and other experimental treatments.

MATERIAL AND TECHNIQUE

Seeds of *Vicia faba* 'Claudia' were sown singly in 3-in. pots in a heated greenhouse. In order to bring about the strongest possible growth of the plumule all lateral shoots were removed as soon as they began to grow. The material for anatomical study was initially selected with some care.

The shoots of *V. faba* bear leaves alternately in two rows, the first and second leaves of the plumule being reduced to small scales which are presumably of little importance as photosynthetic organs. It seems that the conditions in the first and second internodes must therefore be different from those in the succeeding internodes, and the present study begins with the third internode, which is the first one to bear a normal foliage leaf. The third leaf and those immediately following it normally have two leaflets each, together with a small terminal process generally regarded as a vestigial tendril. The third leaf fairly frequently has a terminal leaflet in place of the tendril rudiment, but the occurrence of a terminal leaflet on any leaf other than the third is apparently very uncommon. Plants exhibiting these features have been excluded from the material studied. The upper leaves have more than two leaflets, a condition which generally appears first in the ninth or tenth leaf of the plumule. This change, which is followed almost at once by the initiation of flower-buds, may reasonably be regarded as marking the end of the seedling stage, and the present study is concerned mainly with the internodes which are associated with bifoliolate leaves.

A transverse section of a plumular internode is shown in Fig. 1. The vascular bundles in this section fall into three categories. Firstly there are the two cortical bundles which give off at each node branches which enter the leaf as lateral traces, the cortical strands being replenished by bundles which join them from the central cylinder of the stem. The cortical bundles are of a decidedly anomalous nature, being neither leaf traces nor typical stem bundles, and are therefore omitted from the subsequent discussion. Secondly there are the two large and conspicuous bundles ${}_nM_n$ and ${}_nM_{n+1}$, situated on the diameter at right angles to that which includes the cortical bundles. One of these bundles is the median trace of the next leaf above the plane of the section, the other is the median trace of the leaf above that. Fortunately it is always possible to distinguish between them, since the cortical bundles are always placed so that their xylem inclines towards the median trace of the first leaf above the plane of section. Lastly there are the remaining bundles of the central cylinder. None of these bundles are leaf traces, in the strict sense of the term, but all of them are ultimately connected with the traces of higher leaves, either with the median traces directly or with the lateral traces by way of the cortical bundles.

In order to obtain numerical results it is necessary to find some way of measuring the degree of development which a particular vascular bundle has reached. The number of methods which are available for this purpose is limited. Almost the only practicable possibilities are the counting of cells and the measurement of areas. The method adopted, after a few preliminary trials, was to count the number of fully lignified and apparently functional cells in the primary xylem as seen in transverse section. This procedure has the advantage, as compared with a measurement of area, of taking into account the differentiation of the tissue as well as its bulk, besides avoiding the difficulties arising from the variable and irregular shapes of the bundles. It is, however, very far from being an ideal form of measurement. The nature of the cells counted is left in doubt. In the younger bundles they are probably all vessels, but in the older bundles other types of element are certainly included. Also, owing to the obliteration of protoxylem elements which can then no longer be counted, there is at least a possibility that at some stage in the development of the bundle there may actually be a decrease in the cell-count. Fortunately this last objection appears to be of theoretical importance only.

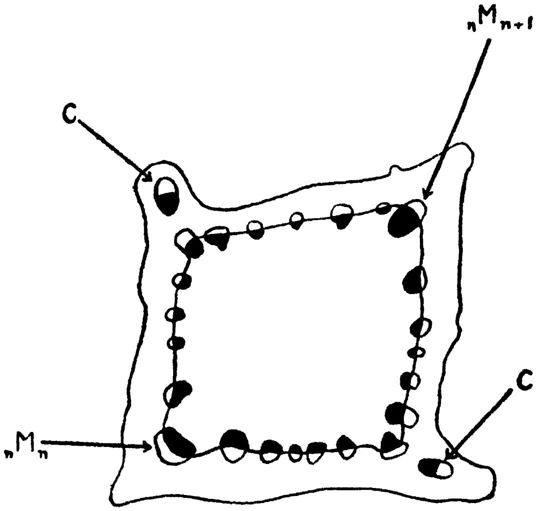


FIG. 1. Transverse section of a plumular internode of *Vicia faba*. $_nM_n$, median trace of first leaf above the plane of section; $_nM_{n+1}$, median trace of second leaf above the plane of section; *CC*, cortical bundles.

It will be convenient to have a standardized system of symbols for the various cell-counts which are to be discussed. Taking a section of the *n*th internode, we may write $_nM_n$ for the count of the median trace of the *n*th leaf, and $_nM_{n+1}$ for that of the median trace of the $(n+1)$th leaf. The remaining bundles of the central cylinder cannot be dealt with individually, since they are extremely variable in number and arrangement, but we shall have occasion to consider their total cell-count, which will be denoted by T_n.

Throughout the investigation the sections have been cut freehand from about the middle of the internode and stained with phloroglucin and hydrochloric acid, the cells being counted under a $\frac{1}{6}$-in. objective. In leaf traces the cells were generally counted individually, but *T* is often so large that an actual count is hardly practicable. In such cases an estimate has been made by counting the cells in one of the larger bundles and then judging by inspection the factor by which this figure is to be multiplied to give the value of *T*.

THE MEASUREMENT OF AGE

One of the principal difficulties in all work of this kind arises from the lack of any natural scale by which to assess the age of the material. It is utterly useless to group the observations according to the chronological age of the plants. Not only is the rate of growth dependent on temperature and other conditions to such a degree as to make it impossible to compare two series of observations made at different times, but the variability of individual plants is so great that plants of the same chronological age may have reached very different stages of morphological differentiation, while plants which are structurally similar may be of quite different chronological ages. These differences are brought about by unavoidable unevenness in speed of germination, slight differences in the depth at which the seed is sown, and so on. What is needed is some criterion by which to judge the 'physiological age' of the material, in the sense in which this term is used by Ashby and others. In the present investigation this problem arises in two distinct forms. It is necessary to have not only a measure of the physiological age of the plant as a whole, but also a means of expressing the physiological age of a single internode, since the internodes of a plant are obviously all of different ages.

So far as the physiological age of a single internode is concerned, it has for the present purpose been assumed that the observed value of *T* is a sufficiently accurate measure. This assumption is justified by the fact that *T* represents the sum of the cell-counts of all the bundles serving the upper part of the shoot, and therefore increases as the internode in which *T* is measured becomes farther and farther removed from the apex. So long as the observations are confined to the counting of cells there is really no other criterion available, and there is no obvious reason why estimates obtained bv other methods should be more truly representative.

The physiological age of a plant as a whole has been determined by using the time scale provided by the formation of successive leaves at the shoot apex. This scale, the interval of which is the plastochrone, is generally accepted as the appropriate scale for studies on shoot differentiation. In applying it to the purposes of the present investigation the procedure has been to count up the shoot to the highest leaf which is fully unfolded or shows at the most a slight rolling of the extreme edges. The serial number of the next leaf is taken as the age of the plant in plastochrones. Thus, if the age of a plant is recorded as 8, this implies that the plant has five fully expanded foliage leaves (numbers 3–7 inclusive), leaf 8 being in an immature condition and not yet fully open. This scale of time is obviously a crude one, since there is no way of estimating fractions of a plastochrone. Attempts to distinguish finer subdivisions in the unfolding of a leaf have not so far been very successful. A mathematical treatment will be given elsewhere by which this difficulty may perhaps eventually be circumvented in certain cases. Unfortunately the observations which are described here do not fulfil the required conditions, and no simple mathematical treatment can at present be applied to the bean plumule. In view of its lack of precision the plastochrone scale has been used as little as possible, and the discussion is mainly based on the use of *T* as an index of the physiological age of single internodes.

SOURCES OF ERROR

It is evident that the cell-counts are themselves subject to errors of several kinds. Apart from mechanical slips such as counting the same cell twice or dropping one of the groups of twenty in which the counting was carried out, there is considerable room for errors of judgement in deciding which cells are to be included in the count and which are to be excluded on grounds either of immaturity or of obliteration. As, however, all the cell counts have been carried out by the same observer working under standard conditions, there is no reason to suppose that these errors have any important systematic component. The same may be said of the procedure for estimating the larger values of *T*. Although individual readings may be in error, the average of several observations is probably fairly reliable.

Another possible source of error is the variation of cell-numbers along the length of an internode. Nothing is certainly known concerning the existence or magnitude of such variations, and even if one had a very full knowledge of them it is difficult to see how any correction could be made, since no method is available for finding equivalent points in internodes of different lengths. The only precaution which it has been possible to take is the obvious one of cutting all the sections from the middle portions of the internodes, so as to avoid any disturbances associated with the nodes.

A more subtle kind of error arises from the use of *T* as an index of physiological age. It is obvious that of two corresponding internodes belonging to different plants, one may at every stage of its development yield a higher cell-count than the other, owing to an inherent difference in vigour between the two individuals. But as the cell-count is used as a measure of age, the more vigorous plant will, quite wrongly, be grouped with physiologically older plants than the less vigorous one. Variations due to age are thus inextricably confused with those due to inherent differences in vigour. The consequences of this confusion cannot be foreseen, but it is probable that no simple statistical treatment of the observations can be strictly valid.

GRAPHICAL METHODS

The variability of the material is so great that the direct plotting of single observations results in the points of a graph being scattered over a considerable area. The probable form of each graph could, of course, be determined by calculating a multinomial regression equation. In view, however, of the uncertainties arising from the use of T as a measure of physiological age, it seems likely that the appearance of accuracy resulting from the calculation of regression coefficients to several significant figures would be dangerously misleading, while the magnitudes of the coefficients would not in themselves be of any particular interest. On the other hand, the use of 'eye-fitted' curves introduces a subjective element which is highly objectionable. A mechanical method of curve-smoothing has therefore been employed. The data are arranged in numerical order of the independent variable, and the resulting table is divided into sets of five observations. At the end of a table three or four observations are allowed to form a set, while if there are only one or two observations left they are added to the preceding set. Both variables are then averaged for each set, so that each point of the graph, except perhaps the last one, is based upon five observations. This operation is absolutely free from any personal bias, its principle effect being a slight flattening of all the curvatures of a graph, bringing it artificially nearer to a straight line than is really the case. The same procedure has been used for all the graphs except those in Fig. 2, where the scale of abscissae is naturally discontinuous.

In plotting graphs of M against T, both variables have been plotted logarithmically. This method has no particular theoretical significance, but is employed simply as a convenient means of magnifying the earlier part of a curve at the expense of the remainder.

THE BEHAVIOUR OF T

The continuous curves of Fig. 2 illustrate the way in which T changes with the increasing plastochrone-age of the plant. It is clear that in each internode T at first increases very slowly, but afterwards at an increasing rate. It is perhaps not immediately obvious that the behaviour of T is significantly different for every internode, but a careful examination reveals two important trends. The upper broken line shows for each internode the value of T in that internode during the unfolding of the associated leaf. In order to show this curve on a reasonable scale all ordinates have been multiplied by 20. This curve shows a rapid fall from the third internode to the fifth, followed by a rather more gradual rise. During the unfolding of the third leaf there are about 22 vessels serving the shoot apex, whereas during the unfolding of the fifth there are only about 6. The latter figure seems astonishingly small, since these 6 vessels, together with an unknown but certainly quite small number in the cortical bundles, must supply the whole of the necessary water for the apical meristem and for the associated leaf-primordia, some of which are of considerable size. It is obvious that this attenuation of the vascular supply to the apical region cannot continue indefinitely, and in fact the graph shows a rise in the number of vessels serving the meristem until at the unfolding of the ninth leaf there are 16.

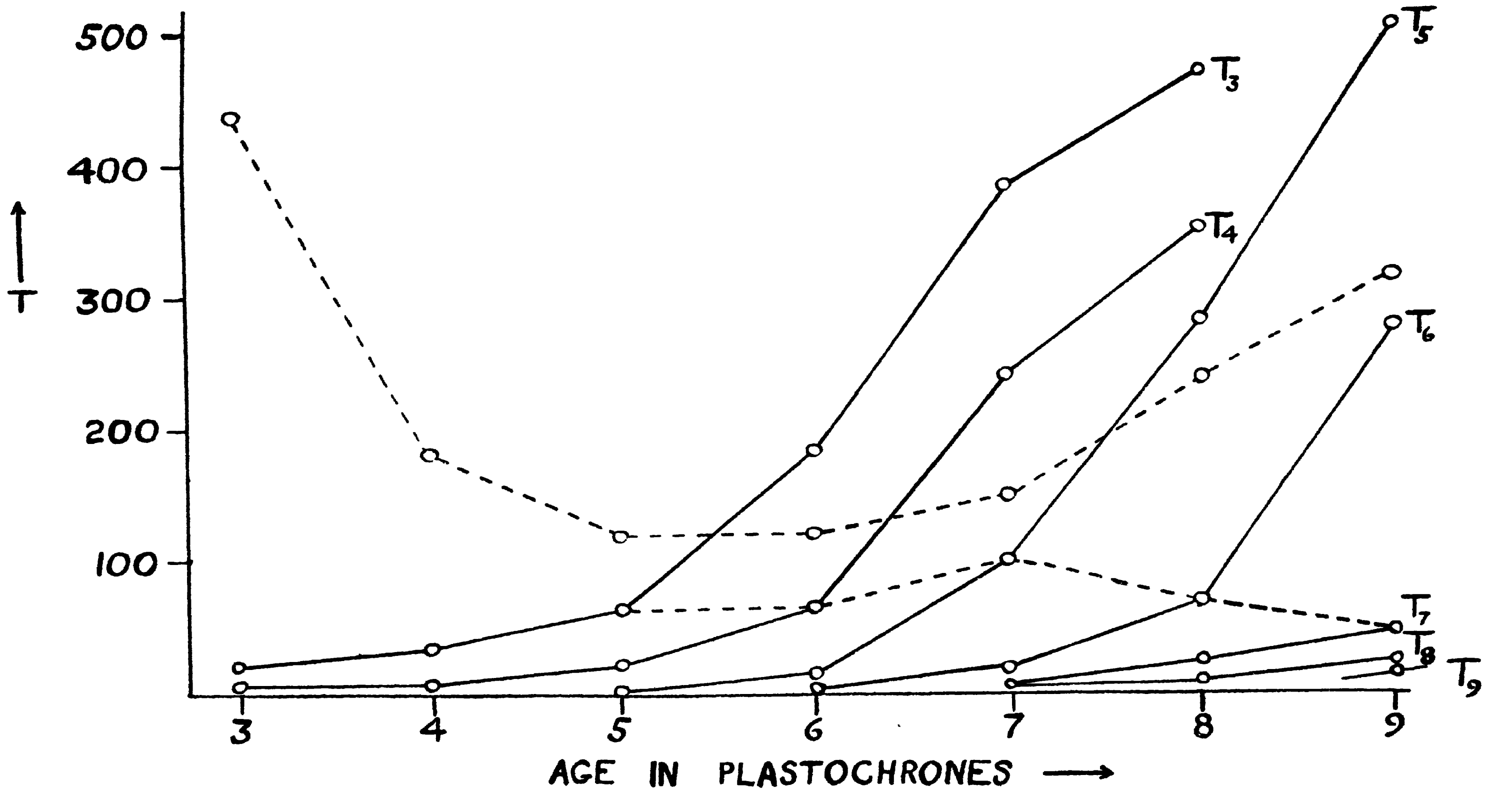


FIG. 2. Continuous lines are graphs of *T* against plastochrone-age of the plant. Upper broken line shows the value of *T* for each internode at the time when the corresponding leaf unfolds (in drawing this curve ordinates have been multiplied by 20). Lower broken line shows value of *T* for each internode at the time when that internode ceases to elongate.

Less conspicuous, but quite as important, is the change in the gradient of the curves of T as one progresses from the third internode towards the upper part of the shoot. The graph of T_4 is a little steeper than that of T_3, and that of T_5 is steeper still, but the internodes above the fifth show a falling-off in the steepness of the curve. This implies that the development of the fourth and fifth internodes follows more closely upon that of their predecessors than is the case for later internodes. This effect may be demonstrated more clearly by plotting the values of T for each internode not against the age of the plant as a whole, but against the value of T for the preceding internode. When this is done it is found that each internode yields a curve of characteristic form. In Fig. 3 are shown the curves obtained by plotting T_{n+1} against T_n for the lower internodes of the plumule. These curves show very plainly that the development of the fourth internode follows upon that of the third more closely than that of the fifth does upon the development of the fourth, and so on. Another point brought out in a striking manner by these curves is the fact that these differences between successive internodes become apparent mainly in the later stages of development. During the early stages, when T is less than about 50, the development of every internode is related to that of its predecessor in approximately the same manner.

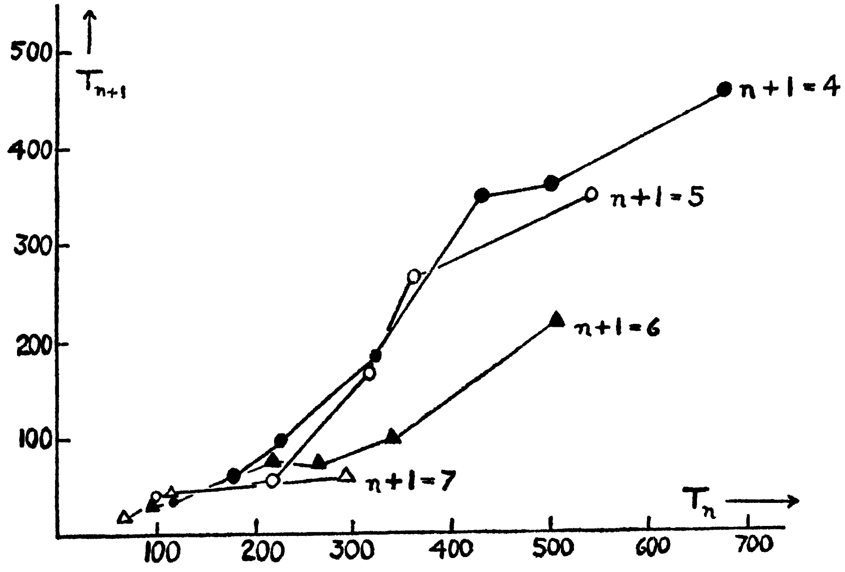


Fig. 3. Graphs of T_{n+1} against T_n.

It is a matter of some interest to determine what is the value of T at the time when the internode ceases to increase in length. For this purpose a considerable number of rapidly growing plants of various ages have been marked with indian ink by means of a simple device which makes two transverse lines about 3 mm. apart on each internode to which it is applied. The plastochrone-age of the plant is recorded at the time of marking. After 2 or 3 days the marks upon the stems are compared with the marker that made them. Although there is a certain amount of variation, most of the observations agree in that the longitudinal growth of the nth internode is found to have ceased during the unfolding of the leaf $(n+2)$. The relevant values of T are indicated by the lower broken line in Fig. 2. The various internodes yield values lying approximately in the range 50–100, with an average of about 70. Owing to the unsatisfactory nature of the plastochrone time scale the more exact determination of the critical value of T would be an undertaking of some difficulty. There is no satisfactory evidence for any variation in the value from internode to internode. It is, however, rather striking that the value of T when elongation ceases does not markedly differ from that at which the curves given in Fig. 3 first begin to separate.

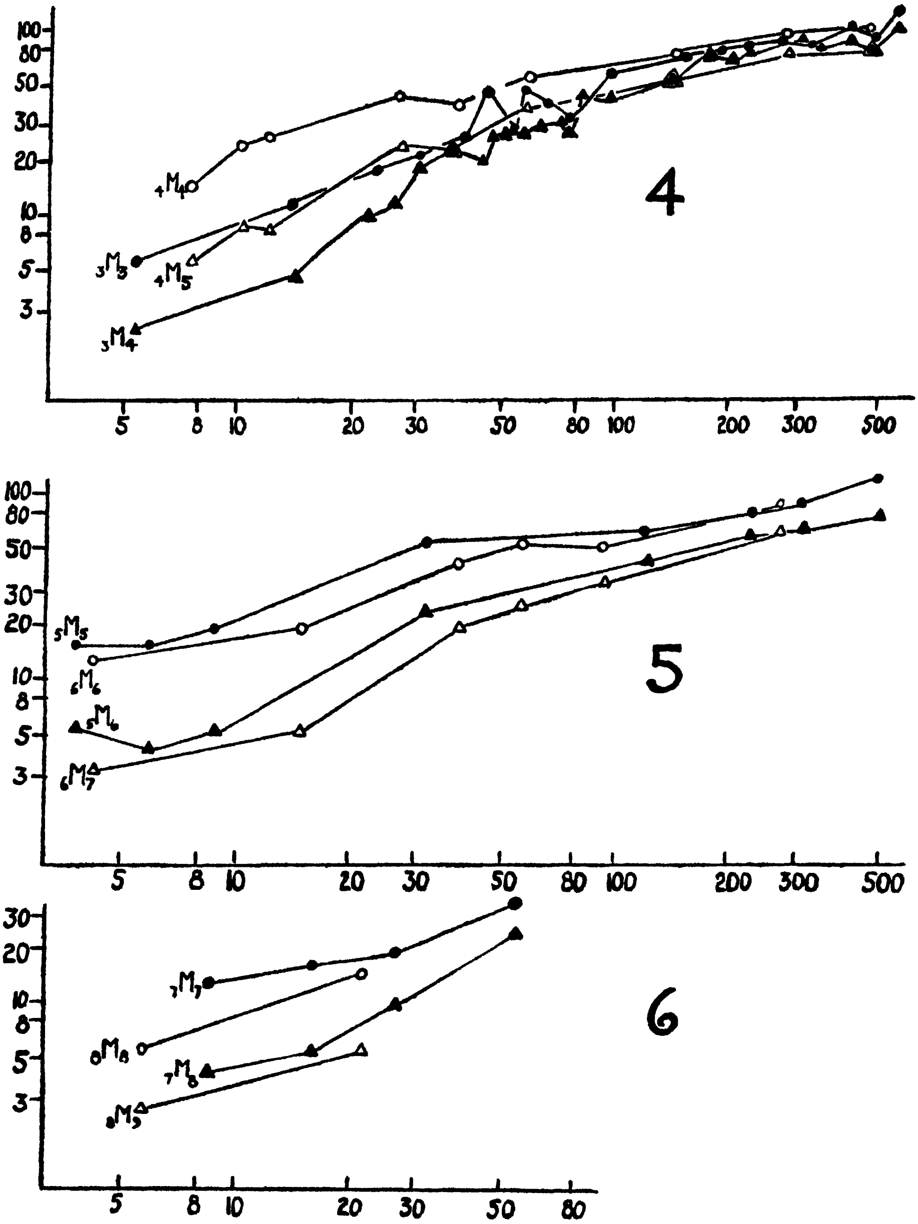
THE BEHAVIOUR OF M

In order to avoid the use of the inaccurate plastochrone scale, the variation of M has been studied with reference to the corresponding values of T. The phenomena are best represented by plotting graphs of ${}_nM_n$ and ${}_nM_{n+1}$ against T_n. For purposes of comparison it would be convenient to have all the graphs for the internodes investigated plotted on the same axes. Unfortunately this is hardly practicable, owing to the crowding which would result. The curves have therefore been divided into three groups. Fig. 4 gives the graphs for the third and fourth internodes, Fig. 5 those for the fifth and sixth, and Fig. 6 those for the seventh and eighth.

A comparison of all these curves shows an important trend as one passes up the shoot from the third internode to the eighth. From an inspection of Fig. 4 it will be clear that the curves for the fourth internode run considerably above the corresponding ones for the third internode. Although the reader will only be able to verify the fact by making measurements, the curves for the fifth internode are almost exactly of the same form as those for the fourth. It will be found from Figs. 5 and 6 that there is then a steady fall of the curves with each successive internode, until internode 8 is reached, which yields curves almost identical with those for internode 3.

These results may be expressed in either of two ways. Either it may be said that the development of the leaf traces is relatively tardy in the third internode, is quicker in the fourth and fifth, and becomes gradually slower in the subsequent internodes; or it may be said that the development of the other bundles in the stem is hastened in the third internode, relatively delayed in the fourth and fifth, and speeded up again in the internodes above. The form of expression adopted will depend on which time-scale is chosen as the more fundamental—the one based on the development of the leaf trace, or the one based on the development of the internode as a whole.

It is obviously a matter of some interest to determine what is the number of vessels in the median trace of a leaf at the time when that leaf is unfolding. This can easily be done by taking from Fig. 2 the values of T indicated by the



FIGS. 4–6. Graphs of M against T, both variables plotted logarithmically.
Fig. 4, internodes 3 and 4; Fig. 5, internodes 5 and 6; Fig. 6, internodes 7 and 8.

upper broken line and reading off the corresponding values of M from the curves of ${}_nM_n$ given in Figs. 4–6. (In a few instances a slight extrapolation is required.) This method will, of course, give the number of vessels in the upper part of the trace. To determine the number in that portion of it which lies in the lower internode of the two which it traverses we must proceed in a rather different manner. Reading off from the graphs of Fig. 2 the value of T_n at the time when the leaf $(n+1)$ is unfolding, the corresponding value of ${}_nM_{n+1}$ is found from the appropriate curve of Figs. 4–6. The results of these investigations are set out in Table I, and show that there is, as one traverses the stem upwards, a steady decline in the number of vessels in the median trace of the leaf at the time when the leaf unfolds. This decline is more marked in the lower part of the leaf trace than in the upper part. The numbers of vessels are all surprisingly small. The leaf as it unfolds has only some 10 or 15 vessels in the upper part of its median trace and a number not exactly known but probably no greater in its two lateral traces. It would seem on general grounds that the decline in the number of vessels serving the leaf at the time of its unfolding cannot continue indefinitely in the higher part of the stem.

TABLE I

Values of M_n at the Time of Unfolding of Leaf n

n	${}_{n-1}M_n$	${}_nM_n$
3	—	16·6
4	21·8	16·6
5	20·8	14·5
6	11·5	13·8
7	13·8	11·7
8	10·0	10·9
9	6·3	—

There is yet another way of studying the variation of M. It has already been shown that the upper and lower part of the same median trace may behave differently, and it is obviously of some interest to find how the development of the upper part is related to that of the lower. This would be most conveniently done by plotting graphs of ${}_{n+1}M_{n+1}$ against ${}_nM_{n+1}$. Unfortunately the graphs obtained from the data of the present investigation are too irregular to be worth publishing. Instead there are set out in Table II the mean values of the fraction ${}_{n+1}M_{n+1}/{}_nM_{n+1}$ for the traces of leaves 4–8 inclusive, within the range investigated. It is clear from these figures that in the median trace of the fourth leaf the development of the upper part keeps pace with that of the lower, but that in the traces of higher leaves the upper part lags more and more behind the lower portion of the same trace. There is a decided tendency for the fraction to be larger in the earlier stages of development, and the slight

TABLE II

$n+1$	${}_{n+1}M_{n+1}/{}_nM_{n+1}$
4	1·016
5	0·976
6	0·859
7	0·654
8	0·720

rise in the value for the trace of leaf 8 is probably to be attributed to this effect, since this trace has only been studied in its younger stages.

DISCUSSION

Sufficient data have been obtained to make it possible to give a fairly detailed account of the development of the xylem of each internode of the bean plumule within the range of this survey. Considering first the general development of the internode itself, without reference to the leaf-traces which traverse it, we see that in the earlier part of its history, up to a point considerably beyond the stage at which the associated leaf unfolds, the internode develops in a manner which bears a constant relationship to the development of the internode below. At about the time when stem elongation ceases, the behaviour of the internode begins to depend on its position in the stem, development being more rapid, relative to that of the preceding internode, in the lower internodes of the plumule. It is tempting to suppose that the marked stimulation shown by the lower internodes at this point may be due to the presence of reserve substances in the cotyledons. It is intended at a later date to carry out experiments designed to test this hypothesis.

Regarding the development of the leaf traces, it is particularly interesting to find that there is no fixed relationship between the development of a trace bundle and that of the internode in which it is situated. Furthermore, the degree of trace development corresponding to the unfolding of the leaf is different for every internode. These facts serve to emphasize the unsatisfactory nature of the plastochrone time scale. It seems perfectly clear that the unfolding of the leaf, unlike the cessation of stem elongation, does not mark any definite epoch in the development of the internode.

The relationship between the upper and lower parts of the same median trace has a direct bearing on the question of the control by the leaf of the development of its traces. It is quite clear that for the leaves above the fourth the lower part of the trace is, at least in the later stages, always in advance of the upper part. This is the reverse of what would be expected if development were stimulated by any influence travelling downwards from the leaf. Whatever may be true of the early stages, the development of the trace after the unfolding of the leaf is markedly acropetal.

The results of this investigation also raise some wider issues. It has been shown that the developing plumule undergoes a complex series of structural changes, each internode being different in various ways from the ones above and below. From the very nature of some of these changes, notably those expressed by the graphs of Fig. 3 and the figures of Tables I and II, it is impossible that they can continue indefinitely in the same direction. There must be a lower limit to the number of vessels serving a leaf at the time when it unfolds, just as there must be a limit to the amount by which the development of an internode can lag behind that of the preceding one. It follows that at some time during the life of the individual these changes must be arrested, possibly reversed. Other measurements undergo a cycle of changes, as shown by the upper broken line in Fig. 2 and by the rise and fall of the curves of M against T in Figs. 4–6. In respect of these characters the higher internodes return approximately to the condition found in the lower ones.

It is impossible to escape the conclusion that all these changes are in some way the result of the alteration in circumstances associated with the transition from a seedling depending on the reserves in the cotyledons to a mature plant independent of such reserves. If this is so, it is to be expected that the changes will not be repeated or indefinitely continued, but that the plant will eventually reach an equilibrium condition in which each internode will be approximately the same as the ones above and below. Even if this expectation is not strictly fulfilled, it is still to be anticipated that there will be some stage at which the influences derived from the seed no longer play any important part in deciding the behaviour of the plumule. The attainment of physiological independence naturally marks an important epoch in the history of the plant and is likely to be associated with characteristic morphological and physiological manifestations in the plumule.

The data at present available give no indication of the attainment by the plumule of an equilibrium condition. There are, however, several reasons for supposing that the period between the ages of 8 and 10 plastochrones must be of special significance in the life of the plumule. During this period all those quantities which have been shown to vary in a cyclic manner regain approximately the values which they had in the third internode. At this time the value of M at the unfolding of the leaf has become so small that it seems hardly possible that it can fall much farther. Of far greater significance, however, is the fact that it is at this time that the relative tardiness of the growth of the upper internodes first begins to operate. An inspection of Fig. 2 will show that T_6 first exceeds 50 when the age of the plant is about $7\frac{1}{2}$ plastochrones. That is to say, at the beginning of the period which we are now considering, the sixth internode has just entered upon that part of its development which is expressed by the characteristic part of the graph of T_6 in Fig. 3. The resulting retardation not only of the sixth internode but also of all those above it must have a profound effect upon the apical region, and in fact growth curves indicate that at this time the rate of increase in height falls off very markedly.

It is therefore of considerable interest that the multifoliolate condition first appears, in most cases, in the eighth, ninth, or tenth leaf, and that the first flower-buds become visible a little later, suggesting that their initiation occurred during this critical phase. It is hoped that it will later be possible to make a more detailed study of plants in this transitional stage of their development.

SUMMARY

1. The development of the primary xylem of the plumule of *Vicia faba* has been studied by counting the numbers of lignified cells visible in transverse sections.

2. During the early stages, while an internode is still elongating, its vascular differentiation is related to that of the internode below in a manner which is essentially the same for all internodes.

3. After an internode ceases to elongate, the further elaboration of its xylem depends on its position in the stem, the lower internodes showing at this stage a stimulation which is not evident in the higher ones.

4. It is tentatively suggested that this stimulation may be due to the presence of reserve substances in the cotyledons.

5. Relative to the general vascular differentiation of the internode, the leaf traces develop more rapidly in the fourth and fifth internodes than in those above or below.

6. The higher the position of a leaf on the stem, the greater is the tendency for the development of the upper part of its trace to lag behind that of the lower part.

7. The time of unfolding of a leaf bears no fixed relationship to the development either of its own traces or of the internode which bears it.

8. The higher a leaf is placed upon the stem, the smaller is the number of vessels in its median trace at the time when the leaf unfolds.

9. The facts of vascular development seem to indicate that the period immediately following the unfolding of the eighth leaf may be of special significance in the life of the plumule.

10. At about this time the first multifoliolate leaves and the first flower-buds appear.

11. It is likely that this is the time at which the plant becomes fully independent of the cotyledonary reserves.

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Experimental and Analytical Studies of Pteridophytes

XVI. The Induction of Leaves and Buds in *Dryopteris Aristata* Druce

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With Plates XV and XVI and twenty Figures in the Text

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I. INTRODUCTION

BY appropriate treatments of the shoot apex of *Dryopteris aristata* Druce, leaf primordia can be induced to arise in positions other than their normal presumptive positions, buds can be induced in what are normally leaf positions, and leaf primordia in bud positions. These experimental data are held to support the hypotheses that the positions in which lateral members arise at the shoot apex and the regulated development of the leafy shoot can be referred to the existence of growth centres and their physiological fields (Wardlaw, 1949*c*). The underlying ideas, based on the work of Schoute (1913), are these: (i) that the extreme apex of the shoot, and also each young leaf primordium, constitutes a growth centre with a surrounding physiological field; (ii) that new growth centres can only originate outside the existing fields; and (iii) that no fundamental metabolic differences exist between shoot and leaf apices, though there may be differences in the extent or intensity of their fields. By isolating particular regions of the apical meristem by vertical incisions, primordia of large relative size are induced, i.e. inhibitory effects of adjacent primordia have been precluded. A result of particular interest is that leaf primordia can be induced experimentally, the induced leaves arising either on the bases of existing primordia of which the apices had been damaged, or in interfoliar, i.e. in bud, positions.

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These observations open up a large and interesting field for further investigation. In the present paper further observations on the induction of leaves and buds are discussed in relation to the hypotheses set out above.

II. METHODS AND MATERIALS

Large apices of *Dryopteris aristata* Druce were prepared for experimental treatment as already described (Wardlaw, 1947, 1949*c*). Small incisions were made in apices as required by means of fine knives. The apices were washed, placed in moist peat, and kept under observation. From embedded materials structural and histological data were obtained. The semi-diagrammatic drawings in the text were made by means of a camera lucida.

Terminology. The terminology used to indicate existing primordia P_1, P_2, P_3 (in order of increasing age) and primordia yet to be formed, I_1, I_2, &c. (in the order of their appearance) is that of M. and R. Snow (1931). I_1, I_2, &c., are also used to indicate primordia which in due course appeared in the experimental materials.

III. EXPERIMENTAL RESULTS

(*a*) *The effect of puncturing* P_1–P_5 *and* I_1–I_3

The basis of this experiment may be briefly explained. In *Dryopteris*, bud positions occur on the shoot a little above the leaf axil, Text-fig. 1; they are more aptly described as being interfoliar. It will be seen that some bud positions occur on the apical meristem at about the same level as the top cycle of leaf primordia (Text-fig. 1), but in the normal development bud formation in these positions is inhibited. On the hypotheses under consideration, the inhibition of buds at the apex may be due in part to the shoot apex but chiefly to the adjacent leaf primordia. It was therefore argued that if (i) the apices of primordia P_1–P_5 were punctured, (ii) the positions I_1–I_3 were punctured or incised so that the development of their physiological fields would be precluded, and (iii) the apical cell was still inactive, as at the beginning of the growing season, conditions would have been established which would admit of the formation of lateral organs in the normal bud positions on the apical meristem. The appearance of such an experimental apex is illustrated in Text-fig. 2. An initial series of experiments carried out along these lines has already been described (Wardlaw, 1949, 1949*c*): the first indication of growth, apart from the formation of scales, was the appearance of small outgrowths on the punctured primordia P_1–P_5, or in interfoliar positions, lateral to P_1–P_5, or axillary to P_6–P_{10} (Text-fig. 3). These small outgrowths, on further development, were seen to be leaf primordia. Some of them occupied what are normally bud positions.

These experiments were undertaken from February onwards, i.e. prior to and at the beginning of the normal growing season for this fern, when the region of the apical cell is still quiescent. When the experiment was repeated, using apices in groups of five, at monthly intervals from April until July, results closely comparable with those in Text-fig. 3 were consistently obtained.

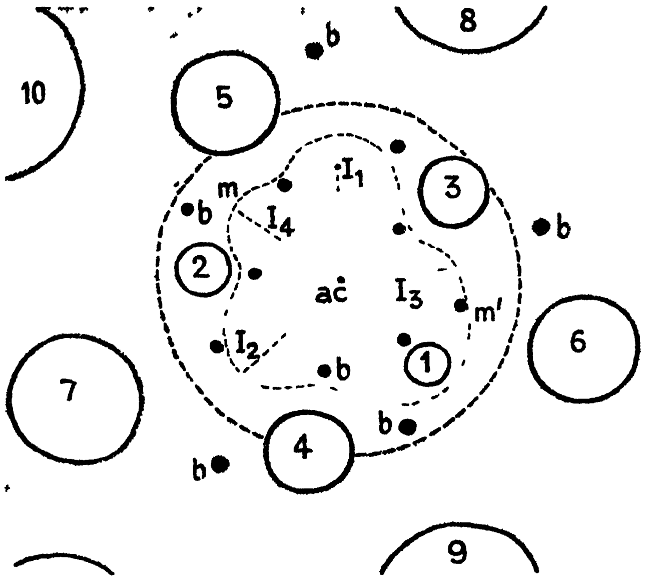


FIG. 1. Apex of *Dryopteris aristata* as seen from above, showing the top cycle of leaf primordia (*P*) 1–5 and the older leaf primordia 6–10. I_1, I_2, and I_3 indicate the next leaf primordia to be formed in the order of their appearance. *b*, the positions of bud rudiments, i.e. bud sites. *m–m* indicates the lower limit of the superficial, prismatic meristematic cells which, together with the apical cell (*ac*) constitute the *apical meristem*. The circular broken line represents approximately the base of the apical cone and the beginning of the broad sub-apical region. (Diagrammatic × 30.)

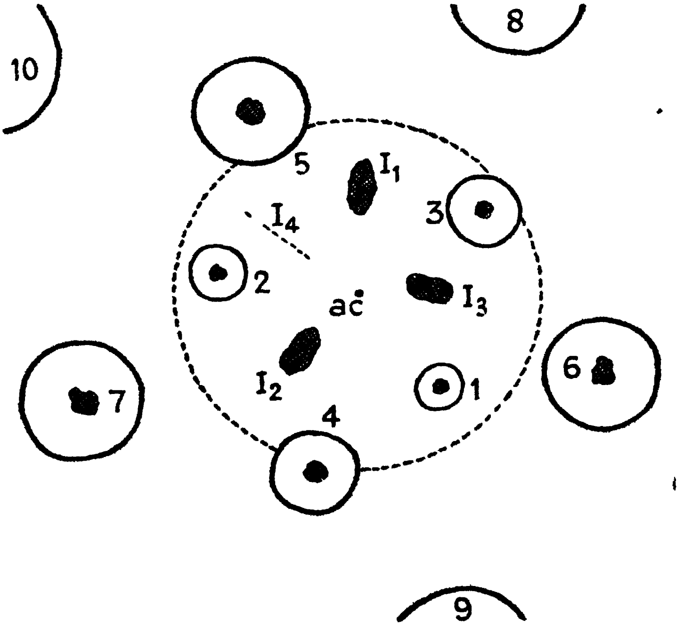


FIG. 2. An experimental apex: the apices of leaf primordia 1–5 have been punctured and also the positions I_1, I_2, and I_3. The position of I_4 is indicated. *ac*, apical cell. (Diagrammatic × 30.) (In the illustrations in this paper, punctured or damaged tissue is cross-hatched.)

The inhibition, in the normal development, of the formation of lateral members between the top cycle of leaf primordia is due, therefore, to the adjacent leaf primordia and not apparently to the extreme tip of the shoot.

In the foregoing experiments both the top cycle of existing primordia, P_1–P_5, and the cycle of primordia yet to appear, I_1–I_3, or I_1–I_4, had been punctured. Further experiments were therefore required to ascertain the part played by these two groups in the normal inhibition of interfoliar members.

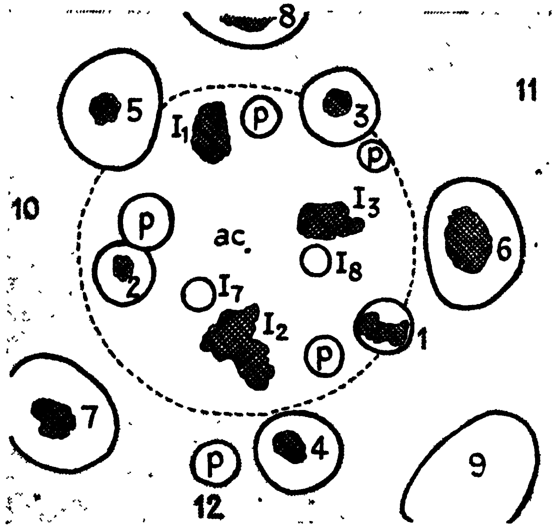


FIG. 3. An apex treated as in Fig. 2, after further growth. Interfoliar leaf primordia (*p*) have been induced between the leaf primordia of the top cycle, i.e. P_1–P_5; some of these induced primordia are axillary to older leaf primordia, e.g. P_8–P_{12}. No new primordia have appeared in the normal I_4, I_5, or I_6 positions, but I_7 and I_8 occupy approximately normal positions. (×27.)

The results of incising P_1–P_5 only, and of I_1–I_4 only, are described in the following sections.

Note. In the writer's view, the essential differences in the morphology of leaves and lateral shoots in ferns, i.e. dorsiventral as compared with radial symmetry, &c., are due to the positions in which they are formed. Since leaf primordia are always formed on the apical meristem, i.e. on the sides of a cone, with a considerably greater rate of growth on the abaxial as compared with the adaxial side, and with possible inhibitional effects proceeding from the apical cell on the adaxial side, the dorsiventral symmetry of leaves appears as a natural consequence. Buds, on the other hand, are typically formed in older, mature regions of the shoot, where this asymmetry of position does not obtain, and they characteristically develop with radial symmetry. If, now, lateral organs are induced to develop in what are normally bud positions on the apical cone about the level of the top cycle of primordia, i.e. P_1–P_5, these organs, for the reasons given above, will have dorsiventral symmetry and will be recognized as foliar members. In this view, leaves and lateral shoots are not specifically determined either by the hereditary constitution of the organism or by the pre-existence in the race of organs of different fundamental categories (Wardlaw, 1949*c*).

(*b*) *The effect of puncturing* P_1–P_5

In several series of experiments primordia P_1–P_5 were punctured and positions I_1–I_4 left intact. In Text-fig. 4 a typical result is illustrated. After some

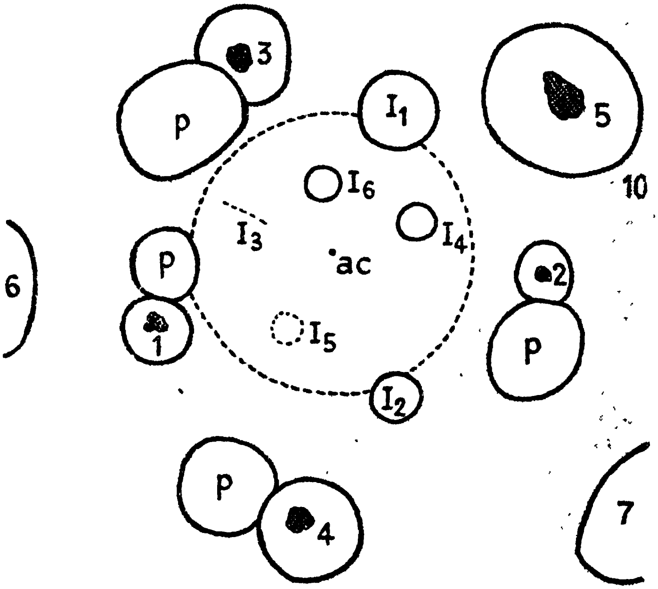


FIG. 4. An apex in which P_1–P_5 were punctured: interfoliar primordia (*p*) have been induced; I_1 and I_2 have appeared in their normal positions; there is no primordium in the I_3 position, but one has formed at I_4; in a later examination the primordium I_5 was observed, in normal phyllotactic sequence with I_4. (× 27.)

time induced leaf primordia could be observed close to or associated with the bases of P_1, P_2, P_3, and P_4. The primordia lateral to P_1 and P_2 are axillary to P_6 and P_7 respectively. The new primordia I_1 and I_2 appeared in their normal positions. On further growth the induced primordia grew rapidly and tended to obliterate the original primordia P_1–P_4. No primordium was formed in the I_3 position presumably because of the presence of the induced primordium axillary to P_6 and lateral to P_3 in that sector. The next primordium to appear, and labelled I_4, is in the normal position for that primordium. The next again, labelled I_6, arose in the sector P_1–I_1, i.e. no primordium had yet appeared in the normal I_5 position in the P_1–I_2 sector. But on further growth a primordium, labelled I_5, appeared in the I_5 position. The position of this primordium is normally determined by I_2 and I_3.

Some apices showed considerably greater growth activity than others. Text-figs. 5 and 6 illustrate apices in which I_1–I_4 were formed after P_1–P_5 had been punctured. In each apex only one induced leaf-base primordium was formed (on the base of P_4, Text-fig. 5, and P_2 in Text-fig. 6). But in each apex an induced interfoliar primordium appeared axillary to P_6, more or less contemporaneously with the formation of I_3, so that the two primordia were observed side by side as illustrated. In other words, an induced primordium arose in that sector of the apical meristem which remained longest without a new normal primordium (i.e. I_3). If I_1 and I_2 are soon formed in their respective positions, and assuming that they exercise a lateral and basipetal inhibitional effect, we should not expect interfoliar primordia axillary to P_8 and P_7 to arise. But as a longer time will normally elapse before I_3 is formed and since, with the continued growth of the apex, the sector P_1–P_3 is expanding tangentially all the time, the induction of a primordium in the interfoliar position P_1–P_3, i.e. axillary to P_6, is what might be expected. Considerations such as these would appear to afford an interpretation of the data of Text-figs. 5 and 6. Several observations of this kind have been made.

The general result of this series of experiments is that the inhibition of interfoliar primordia is due initially and mainly to primordia P_1–P_5, but the next cycle of primordia, i.e. I_1–I_3, may also have some part in this activity.

(*c*) *The effect of puncturing* I_1–I_3

In this series the positions of I_1–I_3 were punctured or incised and primordia P_1–P_5 left intact. If the primordia arising at I_1–I_3 normally exercised an inhibitory effect on the interfoliar primordia of the cycle P_1–P_5, then, in these experiments, by precluding the development of the inhibitory fields of I_1–I_3, some induction of primordia might be expected, especially when the interfoliar spaces have become extended tangentially during growth. Furthermore, the new primordia subsequently appearing on the meristem, i.e. I_4, I_5, and I_6, might be expected to arise close to the adjacent wounds, i.e. those in the I_1 and I_2 positions (Wardlaw, 1949*b*, 1949*c*).

Text-fig. 7 illustrates an apex 4 weeks from the beginning of the experiment. The positions I_1–I_3 were incised but no new primordia, normal or induced, have yet appeared. (Primordium P_4 was injured during one of the periodic examinations and a lateral primordium is beginning to appear.) Text-fig. 8 illustrates a rapidly growing apex after 4 weeks: no interfoliar primordia have been induced in the cycle P_1–P_5, but new primordia have arisen on the meristem above. These constitute part of the normal sequence, i.e. I_4, I_5, and I_6, but all have been formed close to the adjacent wounds (I_1 and I_2). The normal positions of I_4–I_6 are indicated by broken lines. This is the kind of record that has been obtained in eight apices out of ten in this experiment. In Text-fig. 9, I_4 and I_5 were formed close to the I_1 and I_2 incisions respectively, but I_6 occupies a position just above the I_3 incision. In the untreated control apices, two to five new primordia, i.e. I_1–I_5, had been formed during this period, but no interfoliar primordia appeared. In another experiment of

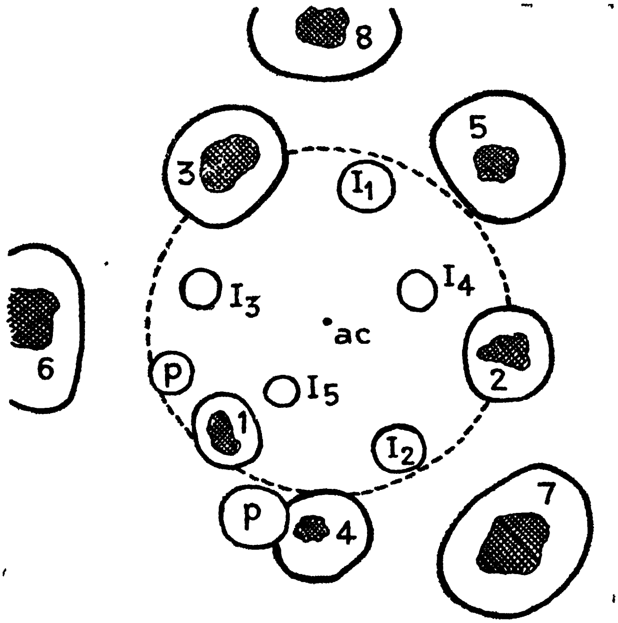


FIG. 5 Experimental treatment as in Fig. 4. An induced interfoliar leaf primordium (p), lateral to P_1, and the new primordium at I_3 have been formed contemporaneously in the same space. (×27.)

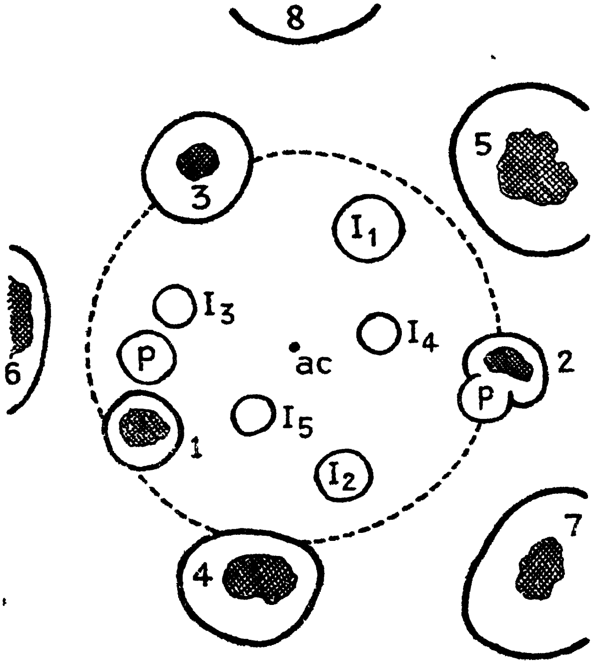


FIG. 6 Another record comparable to that illustrated in Fig. 5. In this apex new primordia have appeared in rapid succession on the apical meristem and only two interfoliar primordia (p) have been induced. (×27.)

this series, in which positions I_1–I_3 were incised, interfoliar primordia were induced in the axils of P_6, P_7, and P_8 in three apices out of five. In these apices primordia only arose at I_4, I_5, and I_6 after a considerable lapse of time, i.e. the meristem was rather inactive. It thus appears that while primordia P_1–P_5 normally inhibit the formation of interfoliar primordia during the first 2–4 weeks, interfoliar members may be induced as the gaps widen during growth, if new primordia are not formed on the meristem above. In the normal development new primordia do appear and their fields preclude the formation of interfoliar primordia in the widening gaps of the cycle P_1–P_5. It is evident that the part played by the apical cell (or the group of cells at the extreme tip of the shoot) must be taken into account in these experiments (see next section).

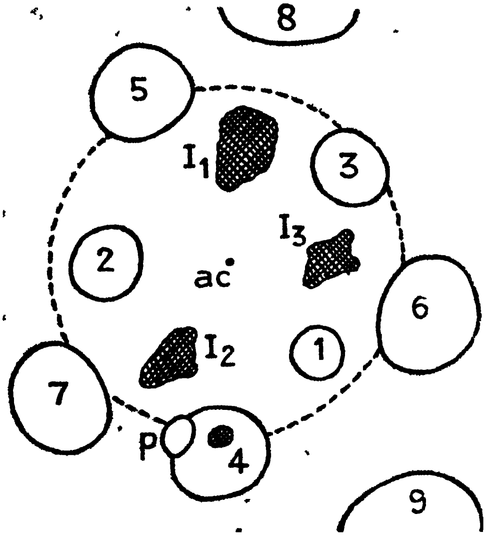


FIG. 7. An apex in which the I_1, I_2, and I_3 positions were punctured, P_1–P_5 being left intact. After 4 weeks, when this record was made, no induced interfoliar primordia had appeared (at P_4, which was injured during one of the inspections, a primordium (*p*) is beginning to form). (× 27)

The general result of these experiments is that where the apex is active and the apical cell and primordia P_1–P_5 intact, interfoliar primordia are not induced in the cycle P_1–P_5.

(*d*) *The effect of puncturing P_1–P_5 and the apical cell*

If the apical cell is punctured, leaf primordia continue to be formed on the meristem until the available space is used up, and buds, axillary to some of the older leaf primordia, are also formed (Wardlaw, 1949*a*, *b*). Also, if a tangential incision is made just above the I_1 position, a bud arises there, i.e. in what is normally a leaf position (Wardlaw, 1949, 1949*c*). In the first instance mentioned above, the apex undergoes further growth: parenchymatous tissue

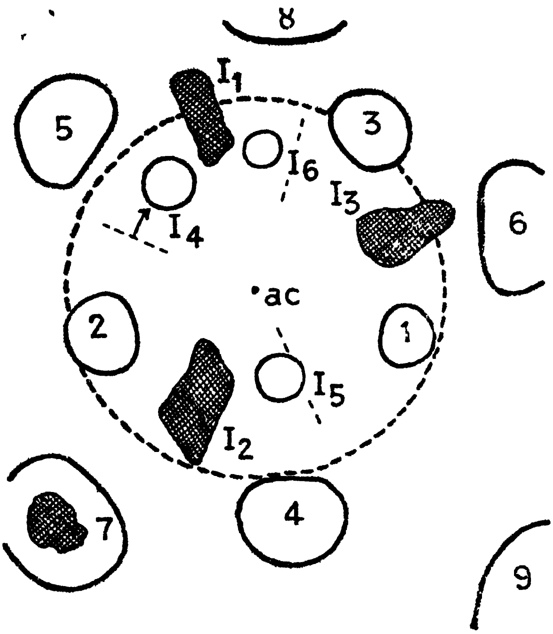


FIG. 8 Experimental treatment as in Fig. 7 no interfoliar primordia have been induced and I_4, I, and I_6 show characteristic displacement towards the wounds at I_1 and I_2 (× 27)

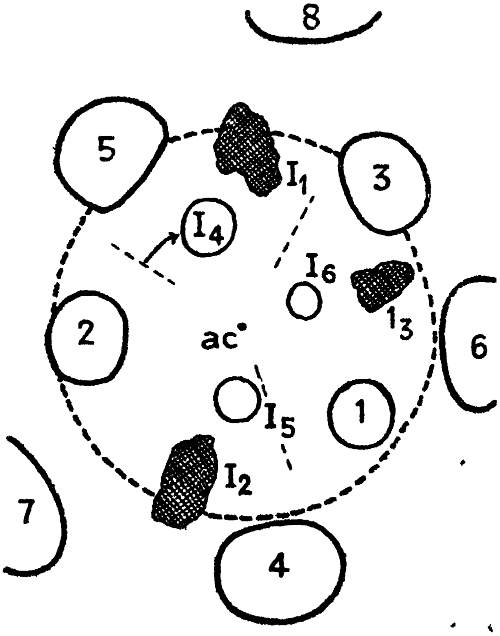


FIG. 9 Experimental treatment as in Figs 7 and 8 no induced interfoliar primordia have appeared, I_4 and I_5 have appeared close to the wounds at positions I_1 and I_2, but I_6 occupies a position between P_1 and I_4 (× 27)

develops in the necrosed apical cell region, the subapical region enlarges, and what was originally the apical cone becomes somewhat flattened out. It is from superficial meristematic cells on this flattened region that buds arise.

In view of the results set out in the preceding sections, it was a matter of interest to ascertain what would happen if the shoot apical cell and the apices of P_1–P_5 were punctured at the same time. Some of the records obtained are illustrated and described below.

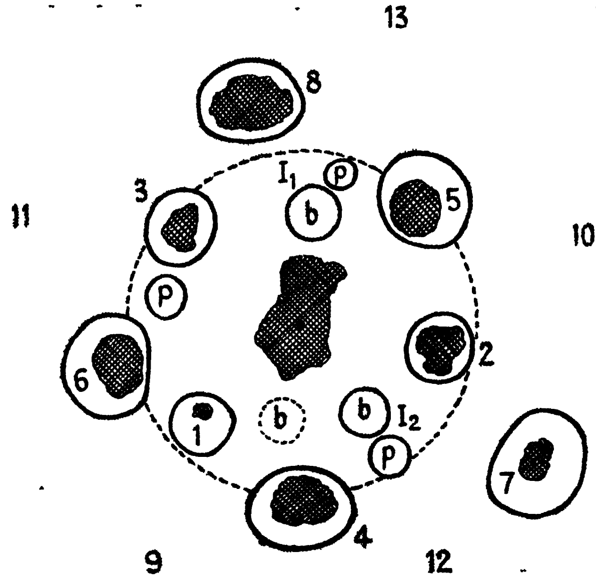


FIG. 10. An apex in which primordia P_1–P_5 have been punctured and also the apical cell. Interfoliar primordia (*p*) have been induced above the axils of P_{11}, P_{12}, and P_{13}, buds (*b*) have appeared in the I_1 and I_2 positions and above the axil of P_4 (× 27)

In the apex illustrated in Text-fig. 10, the first evidence of primordium formation was in the cycle of P_1–P_5; these were leaf primordia and could be interpreted as lying above the axils of P_{11}, P_{12}, and P_{13}. Soon after, shoot buds were observed in the I_1 and I_2 positions and later a further shoot bud was observed above the axil of P_4. In Text-fig. 11, interfoliar primordia have appeared in association with P_2, P_3, and P_4; and whereas a leaf primordium has been formed at I_1, a bud has arisen in the I_2 position; a bud has also been formed in the axil of P_6. In Text-fig. 12 the position of I_1 was damaged and has become necrosed, various interfoliar leaf primordia (*p*) have been induced, and buds have been formed in approximately the I_2 and I_3 positions (or perhaps they are axillary to P_7 and P_6 respectively); the primordium in the axil of P_5 was also a bud. Text-fig. 13 also shows various induced interfoliar leaf primordia (*p*), a bud with its first leaf primordium in the normal I_1 position, a leaf primordium in the normal I_2 position, a bud in the I_3 position (or perhaps it is axillary to P_6), and a bud basal to P_1. Text-figs. 14 and 15 show the same apex on two successive weeks. Induced interfoliar leaf primordia are

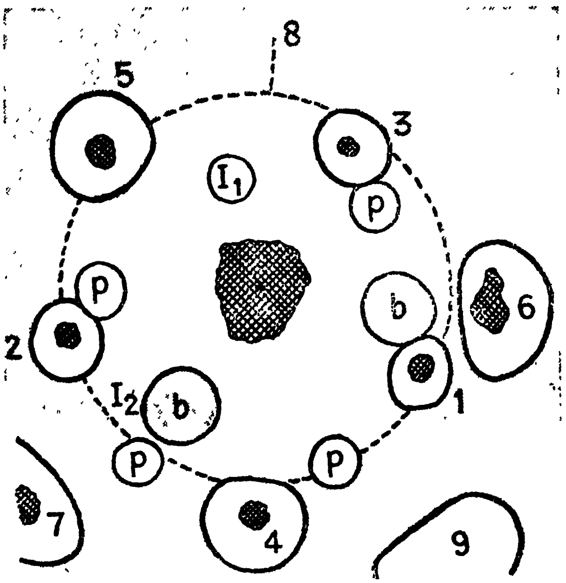


FIG. 11. Experimental treatment as in Fig. 10: some interfoliar primordia (*p*) have been induced; and whereas a leaf primordium has been formed in the I_1 position, a bud (*b*) has been formed in the I_2 position; a bud (*b*) was also formed subsequently above the axil of P_6. (×27.)

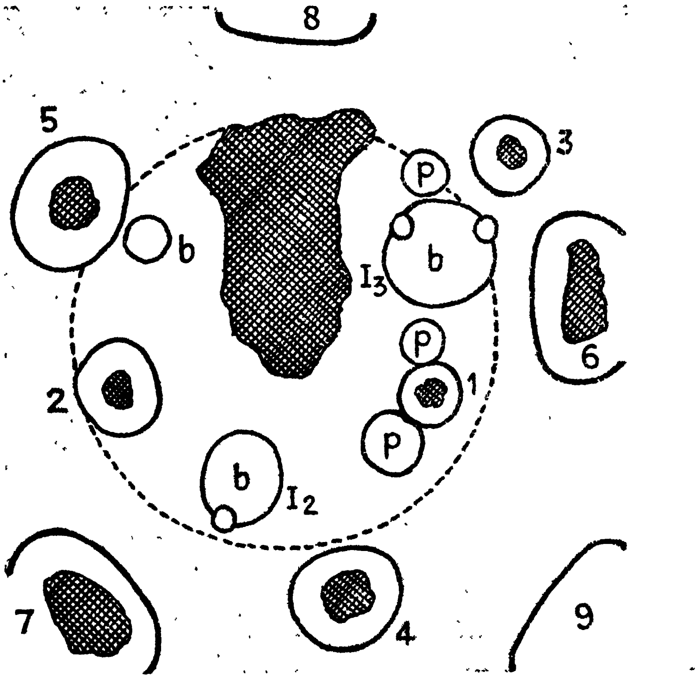


FIG. 12. Experimental treatment as in Fig. 11, the I_1 position being also damaged and necrosed. Induced leaf primordia (*p*) and buds (*b*) with their first leaves have appeared. (×27.)

associated with P_1 and P_2 (Text-fig. 14), a leaf primordium has appeared at I_1, a bud close to the I_2 position, and a leaf primordium at I_3. In Text-fig. 15 these observations are confirmed, but a bud has now arisen above the axil of I_1 and another lateral to P_1 and roughly above the axil of P_6.

At this stage it is not possible to offer any adequate explanation of these several organogenic developments. The induction of interfoliar primordia in the cycle of P_1–P_5 is in agreement with the findings already set out in section III (*a*). The formation of buds in what are normally leaf positions (i.e. at I_1, I_2, or I_3) when the action of the apical cell is removed or precluded has also been the subject of comment (Wardlaw, 1949, 1949*c*). But two features in these records do present special problems. One is that two growth centres, whether of leaf or bud primordia, can apparently originate close together or fairly close together, contemporaneously. The other is that, on the same apex, whereas at one presumptive leaf position a leaf primordium may arise, as in the normal development, at another presumptive leaf position it is a bud primordium that is formed. From this it may be inferred that, in the ferns, quite small differences in the incidence of morphogenetic factors in the apical meristem may determine whether it is a leaf or a bud primordium that is formed. Since the developments under consideration have been obtained when the apical cell was punctured, with concomitant disruption of the

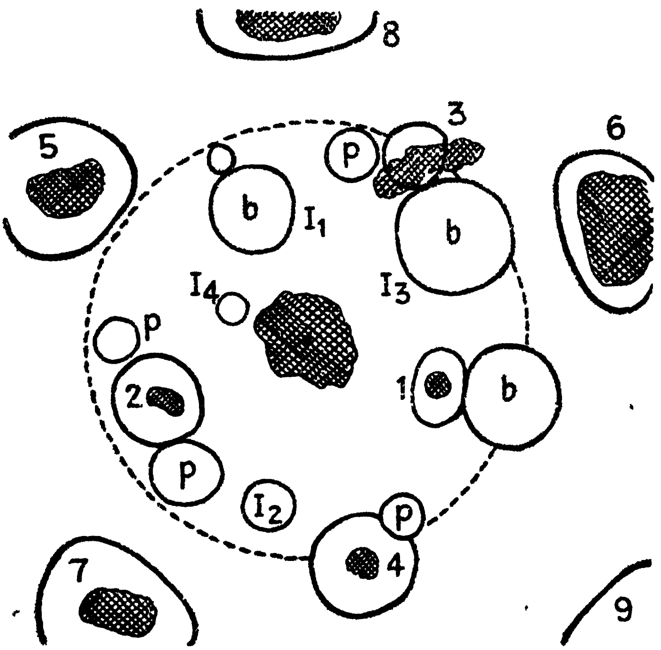
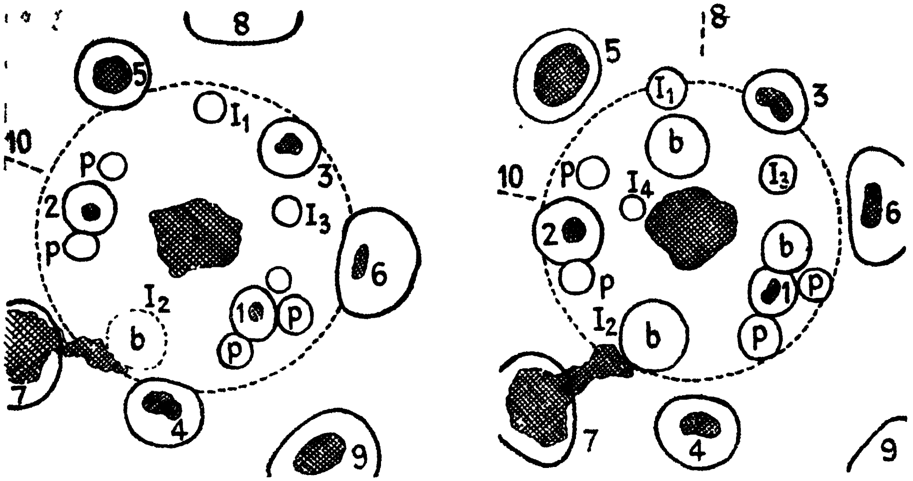


FIG. 13. Experimental treatment as in Figs. 11 and 12 the I_1 position is occupied by a bud (*b*) with its first leaf, the I_2 and I_4 positions by leaf primordia, and the I_3 position (approximately) by a bud, induced interfoliar primordia (*p*) have also appeared. (× 27.)

normal conical construction, it may be tentatively suggested that physical factors and factors determining the distribution of growth are involved.

(*e*) *Minimal space for the formation of a leaf primordium*

According to M. and R. Snow (1931, 1947) a leaf will be formed in the 'next available space' on the apical meristem that attains a certain necessary width and a certain minimal distance below the extreme tip of the shoot. Now, in the ferns, as Text-fig. 1 clearly shows, a new primordium occupies only a small part of the area between two adjacent primordia. Primordium P_1, for example, occupies only a small part of the space between P_4 and P_6. If space on the meristem has the importance attributed to it in the matter of leaf formation, then by curtailing the space between P_3 and P_5, or any other pair of adjacent primordia, the formation of a primordium in that space should be precluded.



FIGS 14 and 15. Experimental treatment as in Fig 13 the same apex is shown on two successive weeks. Various interfoliar primordia (*p*) have been induced; leaf primordia have appeared in the I_1, I_3, and I_4 positions and buds (*b*) in the I_2 position and above the axils of I_1 and P_6 (approx) (× 20)

In the course of these studies, in which the apex has been incised at various points, the formation of a new primordium has been observed in a space of about half the normal size (Wardlaw, 1949*c*). In apices where primordium P_3 and position I_1 were punctured, for example, an induced primordium has arisen in the space between, i.e. in approximately half the normal space as measured by its tangential component.

At the suggestion of Mr. R. Snow, the writer has limited the space available for the formation of a primordium by making two parallel radial cuts close together so as to enclose the I_1 or the I_2 position, with the results illustrated in Text-figs. 16–20. In a number of specimens, where the cuts were so close together as almost to coalesce, primordia were not formed. In other apices, where the cuts were also rather close together (Text-fig. 16), no primordium was formed between the cuts but primordia labelled I_1' and I_2' arose beyond the innermost extremities of the cuts while I_3 and I_4 were formed in their normal positions. This result was frequently obtained. In other apices a primordium originated between the cuts, thus affording evidence of primordium formation in about one-third of the normal 'available' space as measured by the tangential component (Text-figs. 17–20). The illustrations also show that the isolated primordia were characterized by rapid relative growth, as already described (Wardlaw, 1949, 1949*c*). In these experimental materials the knife cuts gape slightly, the exposed tissue becomes brown and corky, while within, in proximity to the wound, there is always some development of parenchyma. Thus, where two parallel cuts are close together, the area of meristematic cells from which a primordium could be formed is small compared with a normal interfoliar area.

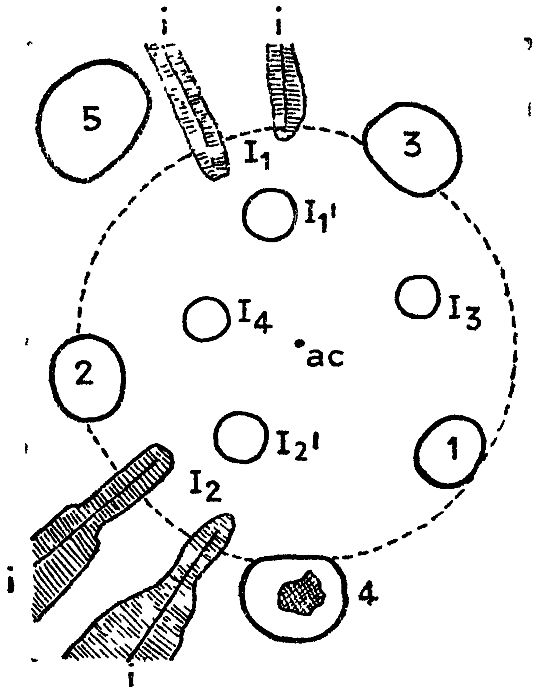
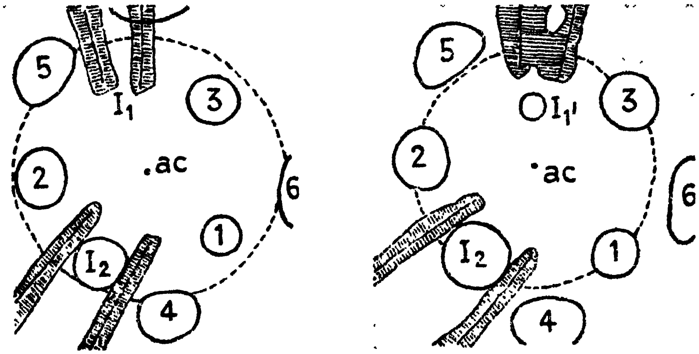


FIG. 16 An apex in which parallel incisions (*i*) have been made so as to enclose the I_1 and I_2 positions new primordia which in due course appeared are shown (× 27.)

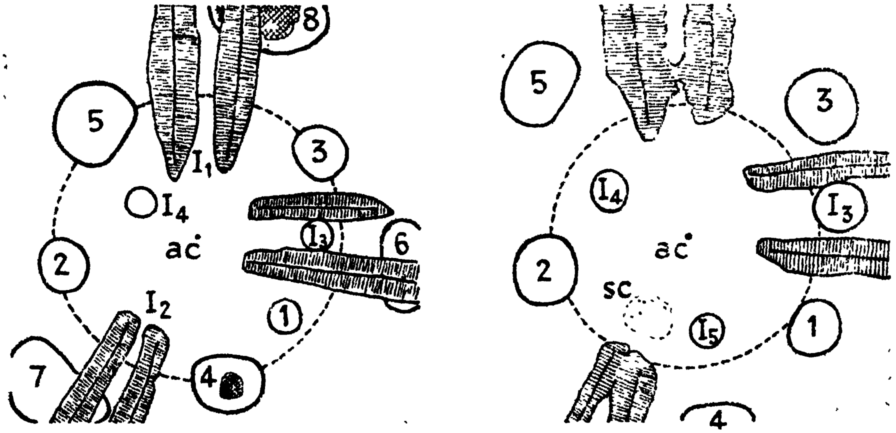
(*f*) *Anatomical observations*

Selected experimental apices were fixed, embedded in wax, and cut in serial transverse sections. Relevant anatomical observations are illustrated in Pls. XV and XVI. The general result of this aspect of the work has been to confirm the external observations illustrated diagrammatically in the text-figures.

Pl. XV, Fig. 1, shows the positional relationships of an induced leaf primordium (*p*) and a displaced leaf primordium (I_4) to an I_1 position which had been punctured; Fig. 2 of this series, taken lower down in the shoot, shows the vascular strands and leaf gaps relating to these two primordia. Observations such as these show clearly that the distribution of vascular tissue in the shoot is determined by the shoot apex and the lateral members to which it gives rise. In the present instance, the positions of the leaf-gaps shown in Fig. 2 are determined by two primordia, one of which (*p*) would not be present in the normal development, while the other (I_4) is displaced from its normal position. Again, in Pl. XVI, Figs. 10–12, the leaf primordium which has arisen



FIGS. 17 and 18. Experimental treatment as in Fig. 16: the same apex is shown on two successive weeks. Fig. 17: A leaf primordium has been formed in the narrow panel of tissue in the I_2 position, but no primordium has yet appeared at or near the I_1 position. Fig. 18: primordium I_2 has shown rapid relative growth and a second primordium, labelled $I_{1'}$, has now appeared; *ac*, apical cell. (× 20.)



FIGS. 19 and 20. Experimental treatment as in Figs. 16 and 17, but incisions have also been made so as to enclose the I_3 position: the same apex is shown on two successive weeks. A leaf primordium has been formed in the I_3 position and has shown rapid relative growth, Fig. 20. Primordia I_4 and I_5 are in their normal positions. *sc*, accidental superficial scar; *ac*, apical cell. (× 20.)

in the I_3 position between two close parallel incisions has an anomalous leaf-trace, i.e. it conforms to the modified outer contour of the shoot and to the space available for development. The highly varied and irregular vascular patterns in apices in which both leaf primordia and buds have been induced lend further support to the view that the initial differentiation of vascular tissue, i.e. its actual inception, is due to the basipetal movement of a substance (or substances) from the growing points of shoots, shoot buds, and leaf primordia (Pl. XV, Figs. 3–6).

The writer has previously shown that a lateral bud in *Dryopteris* and other leptosporangiate ferns typically arises in a position somewhat above the axil of a leaf. This is shown in Text-figs. 10–15 and is confirmed by the anatomical details illustrated in Pl. XVI, Figs. 7–9, 13.

IV. DISCUSSION

Although the investigations described here have brought to light phenomena for which no adequate explanation can yet be offered, the data obtained support the view that growth centres and their physiological fields do exist at the shoot apex in ferns and that the phyllotactic sequence and, more generally, the organization of the leafy shoot and its regulated or harmonious development, can be referred to them. The data, in fact, afford the basis for a unifying conception of primary morphogenetic processes in vascular plants. It may be assumed that the physiological fields associated with the apical group of cells (the apical cell and its adjacent segments) and with the young leaf primordia have many properties, but, from the standpoint of organ formation and position, the inhibitional properties of fields are among the more important. In this connexion the finding of M. and R. Snow that the space on the apical meristem in which the next leaf primordium will arise must be of a certain minimal area receives support, in that this space must be sufficiently large so as not to be completely eclipsed by the inhibitive fields of the adjacent primordia. But the space needed for the site of the primordium may be quite small—a fact demonstrated in the experimental studies described here.

The inhibitional effect of the apical group of cells apparently reaches well down the sides of the apical cone: hence it is that new leaf primordia originate close to the basiscopic margin of the apical meristem. In the ferns, leaf and bud primordia are histologically identical: each originates from a small group of the superficial prism-shaped cells that constitute the apical meristem. At a certain distance below the apical cell, then, any group of these superficial meristematic cells is potentially capable of giving rise to a lateral member. But, in fact, lateral members arise at wide intervals in a regular sequence. According to the present investigations, the position occupied by a new leaf primordium is determined by the fields of the two older primordia adjacent to it and not by all the primordia of the top cycle. This again is confirmatory of the findings of M. and R. Snow. Between the widely spaced leaf primordia lie areas of the apical meristem. Now, why are no lateral members formed in these areas? These interfoliar areas include bud positions, i.e. bud rudiments, but, in the normal development of the shoot, bud formation is completely inhibited. The bud rudiments remain inactive, as far as organogenesis is concerned, and are left behind when the shoot apex grows on. If, however, all the leaf primordia of the top cycle were punctured and the intensity of their fields thereby diminished, the expectation would be that lateral members would arise in interfoliar, i.e. in bud, positions. That this does happen has been abundantly demonstrated during the present investigations. Where the shoot apex is still intact, these induced members are seen to have the symmetry and configuration of leaves. Leaf formation can thus be induced in what is normally a bud position.

The orderly development of the leafy shoot is referable to the continued activity of the apical cell and to the regulated inception of growth centres in the apical meristem. While the leaf primordia of the top cycle preclude the formation of interfoliar members in that cycle, the new leaf primordia which are formed on the apical meristem, i.e. constituting the next cycle above, also play a part in the continued inhibition of the interfoliar members. Here we have evidence of those reciprocal relationships by which the activity of the shoot apex is characterized (Wardlaw, 1948): for whereas the positions and rates of growth of new leaf primordia are determined by the older leaf primordia below—an acropetal effect—the inhibition of interfoliar members is partly due to primordia above—a basipetal effect.

That the fern apex is totipotent in respect of organ formation seems evident from the accumulated data of these studies. In experiments (section III (*d*)) where both shoot and leaf apices were punctured, leaf primordia arose in some of the presumptive leaf positions and buds in others. It thus appears that very little is needed to tip the balance from leaf formation to bud formation. If it be assumed, as has been done here, that there are no fundamental metabolic differences between leaf and bud primordia (i.e. that no special 'leaf-' or 'bud-forming' substances are involved), then the symmetry and configuration of an organ must be determined by the position in which it is formed and by the physical and physiological factors incident in that position. If, on the other hand, it is assumed that specific 'organ-forming' substances are involved, then it becomes necessary to explain how these substances come to be located, at critical concentrations, in particular positions. In the experimental materials under consideration there would be the added difficulty of explaining how, in some instances, a 'bud-forming' substance has become localized in what is normally a leaf site and vice versa. Any general hypothesis of the kind that leaves inhibit leaves and buds inhibit buds—though it offers attractive possibilities—is confronted with the same difficulties. Hence, as it seems to the writer, it is to the mechanics and genic control of growth in the plastic growing region that we must look for an explanation of the characteristic form and structure of the shoot and its lateral members. In the ferns, leaf primordia are formed on the sides of the apical cone in sites which are characterized by marked differences in the rates of growth on the adaxial and abaxial sides:

buds, on the other hand, arise on older regions of the shoot where this marked asymmetry of position and of growth has disappeared. Buds are only induced on the apical meristem after the sites have been isolated from the apical cell or when the latter has been punctured. In both instances the asymmetry of position which characterizes leaf formation has to some extent been removed. No doubt this tentative account of factors which may account for the differences between leaves and buds is an over-simplification, but the data of section III (*d*) show that these differences may not be so great as they appear to be.

According to the growth-centre hypothesis, a new centre can only originate on the meristem outside the effective field of an existing centre. In apices which have been subjected to experimental treatment, the possibility of two growth centres originating simultaneously presents itself and, as the text-figures show, two primordia may arise in close proximity: the development of such primordia and, in particular, their rates of growth merit further consideration.

In the development of the fern plant from the young sporophyte to large adult size, the apical meristem undergoes a progressive increase in size, the apex of the adult being, in general terms, a magnified image of the small apex of the young sporophyte (Wardlaw, 1948). But in an enlarging conical structure such as the apex, certain geometrical relationships are constantly changing, e.g. the actual distance between adjacent leaf primordia increases. Although at present we have no knowledge of the extent or shape of the physiological fields surrounding the leaf primordia in a young sporophyte as compared with those at the apex of an adult plant, nevertheless the probable importance of relevant geometrical aspects is evident. In due course we may therefore expect to see a renewal of interest in the observations and findings of investigators such as Van Iterson (1907).

Interesting as are the results that have been obtained by the methods used here, such an approach has definite limitations. Further investigations of this kind are not precluded, but the next phase of this research clearly lies in the field of the physiologist. It would, for example, be of great interest to know to what substance, or factors of growth, the inhibitional properties of the fields postulated here are due and if there is, in fact, a differential distribution of a growth-regulating substance in the apical meristem. Then, again, if the postulated fields do exist, it seems likely that they will have a three-dimensional aspect, and it may be that the component at right angles to the superficial meristem is effective in bringing about the inception of the vascular tissue which is to be seen immediately below the apical meristem and the organs that originate there. These and related problems would appear to offer a fruitful field for morphologists and physiologists working in conjunction.

On the basis of earlier work on *Dryopteris* and other ferns it was concluded that the position of a bud lies somewhat *above* the leaf axil, not *in* the leaf axil as in flowering plants (Wardlaw, 1943, 1943*a*). The observations on bud formation which have been described and illustrated here fully support the earlier conclusion. From these observations it may be inferred that the inhibitive field of a leaf primordium is inextensive in the acropetal direction, its main effects being lateral and basipetal. If this is so, the inhibition of buds above the axils of the top cycle leaf primordia, i.e. P_1–P_5, will be due to the apical cell group, whereas, as many observations show, the inhibition of buds in interfoliar positions, i.e. lateral to P_1–P_5, is due to these leaf primordia.

The effective physiological field of the apical cell group may be envisaged as extending down the sides of the apical cone as far as the axils of P_1–P_5, the adaxial sides of which show evidence of inhibited growth. An extreme case of this inhibition is seen in *Matteuccia struthiopteris*, where deep axillary cavaties or pockets are formed. It is tempting at this stage to discuss in some detail the shapes of the physiological fields at the apical meristem. Nevertheless, because of the complex nexus of factors involved in the growth of the shoot apex and the many physiological aspects as yet unexplored, it seems advisable to leave the matter as a general statement until further data have been obtained. In zoological literature the field concept has now been elaborated and discussed in very considerable detail by investigators such as Huxley (1935), Weiss (1939), Needham (1942), and others. By comparson, botanists have lagged behind, though one of the first contributions to this subject was made by Gurwitsch (1922) using examples drawn from botanical sources.

V. SUMMARY

1. Further evidence has been obtained in support of the view that the extreme apex of the fern shoot and each young leaf primordium constitutes a growth centre with a surrounding physiological field and that these determine the inception of leaf primordia, the regular sequence in which they appear, and the harmonious development of the leafy shoot.

2. By destroying the leaf primordia of the top cycle, interfoliar leaf primordia can be induced; these occupy what in the normal development would be bud positions (bud formation being normally inhibited). Although the inhibition of these interfoliar primordia is due to the top cycle of primordia, the next cycle of primordia to be formed on the meristem also play a part in maintaining the inhibition.

3. In specimens in which the shoot apex and the apices of the top cycle of leaf primordia were punctured, interfoliar primordia were induced. Of the lateral members which in due course appeared in the I_1, I_2, and I_3 positions, some proved to be leaf primordia and some buds. The apical meristem in ferns is thus seen to be totipotent in respect of organ formation. Small differences in the incidence of physical factors and factors of growth apparently determine whether a leaf or a bud will be formed.

4. A leaf primordium may arise in a half or even one-third of the normal 'available space' between primordia. If a leaf primordium, together with its physiological field, is taken as a morphogenetic unit, the view of M. and

R. Snow that the next primordium to be formed will arise in the 'next available space' is held to be valid.

The writer has pleasure in acknowledging the assistance received from Mr. E. Ashby in microscopic preparations and photographic illustrations.

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EXPLANATION OF PLATES

Illustrating Professor C. W. Wardlaw's paper on Induction of Leaves and Buds in *Dryopteris aristata* Druce.

(All figures are from untouched photographs)

PLATE XV

Fig. 1. An experimental apex in transverse section. The position of I_1 was punctured. An induced leaf primordium (p), axillary to P_8, has appeared laterally; a new primordium I_4 has also been formed in close proximity to the wound at I_1; *vt*, incipient vascular tissue. (× 50.)

Fig. 2. As in Fig. 1, but lower down in the shoot, showing the leaf-traces and leaf-gaps of p and I_4. (× 50.)

Figs. 3 and 4. The apex illustrated in Text-fig. 13 cut in transverse section, showing the complex and greatly modified pattern of the vascular system (*vt*) in Fig. 3, and the approach to a more normal distribution of vascular tissue lower down, Fig. 4. (× 15.)

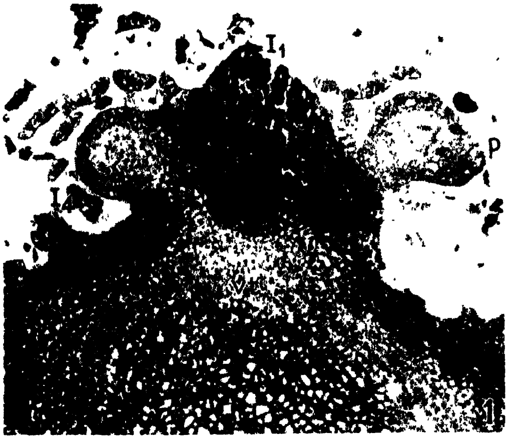
Figs. 5 and 6. Transverse sections of the experimental apex illustrated in Text-fig. 12. The solenostelic vascular tissue (*vt*) of three induced buds (*b*) and the horseshoe-shaped traces of induced leaf primordia (*p*) can be seen; *lp*, leaf primordium, with its axillary bud (*b*). Fig. 6 is closer to the apex than Fig. 5. (× 24.)

PLATE XVI

Figs. 7, 8, 9. The apex illustrated in Text-fig. 15 in transverse section at three levels. The shoot apex (*a*) was punctured; a leaf primordium was formed at I_1, and a bud (*b*) subsequently arose above its axil; the leaf primordium at I_3 is seen in Fig. 9, and also an induced primordium (*p*). (×40.)

Figs. 10, 11, 12. The apex illustrated in Text-fig. 20, in transverse section, at three levels. No leaf primordia have arisen between the parallel radial incisions at I_1 and I_2, but one did arise between the incisions at I_3; the somewhat modified leaf-trace can be seen; *1*, *2*, *3*, &c., the existing leaf primordia in order of age. (×15.)

Fig. 13. Transverse section of the apex illustrated in Text-fig. 11. The apical cell (*a*) was punctured; a bud (*b*) has been formed above the axil of an induced leaf primordium (*p*); other induced leaf primordia (*p*) and the leaf primordium which arose at I_1 can be seen. (×35.)



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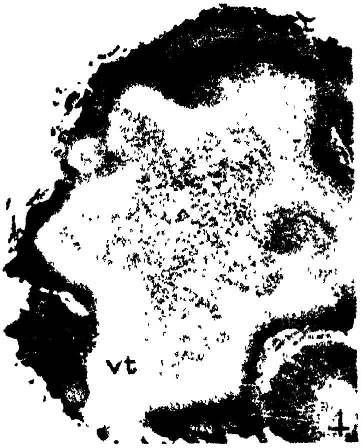

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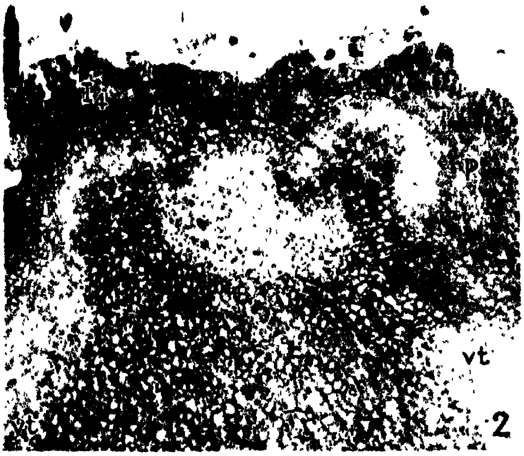

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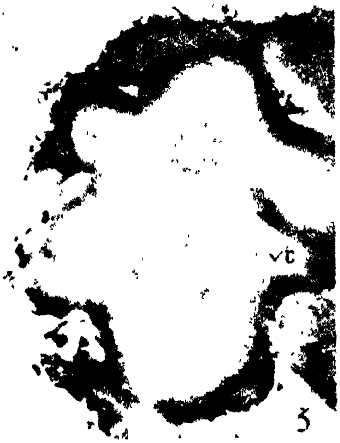

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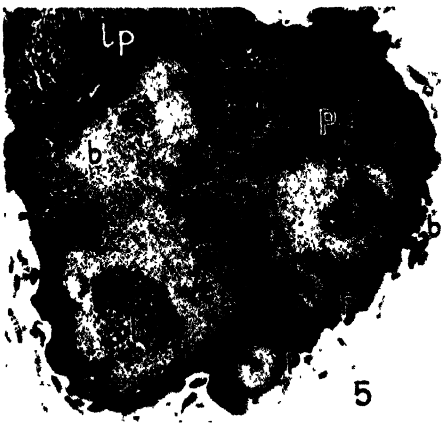

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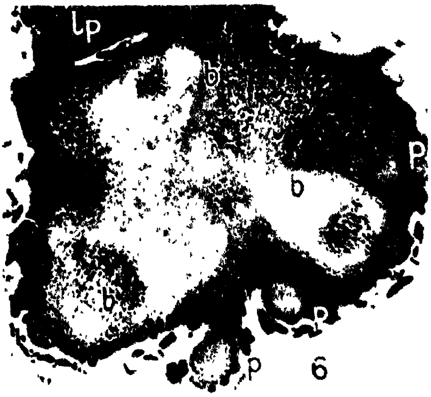

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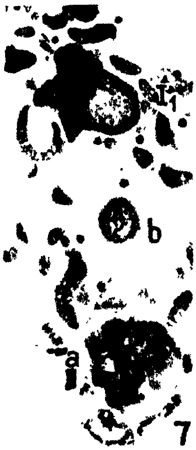
C. W. WARDLAW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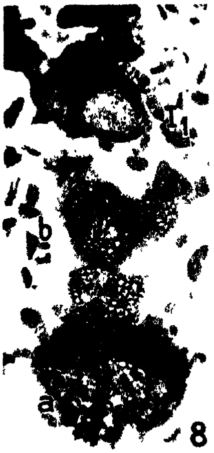
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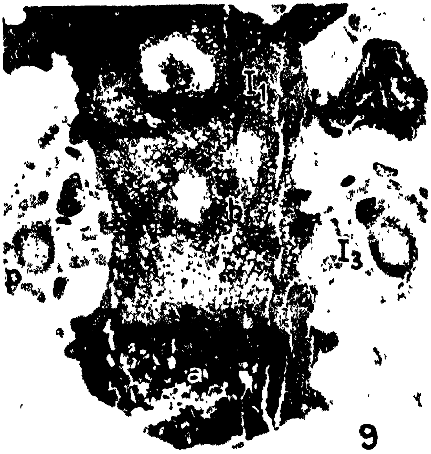
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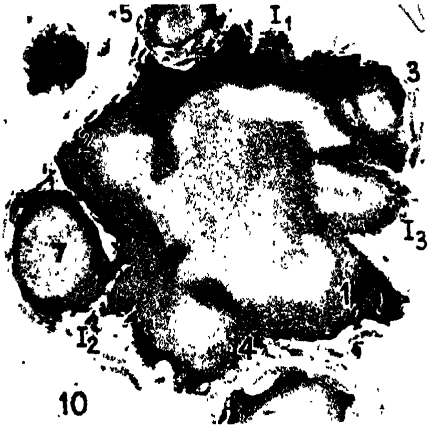
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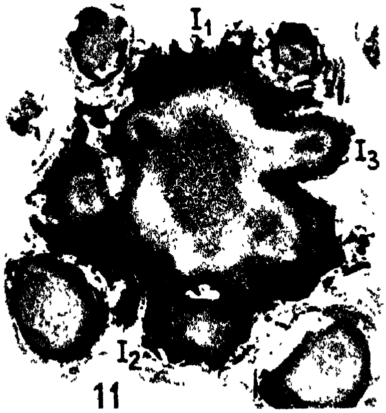
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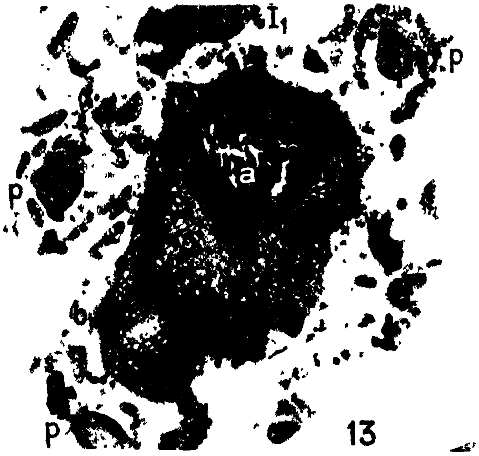
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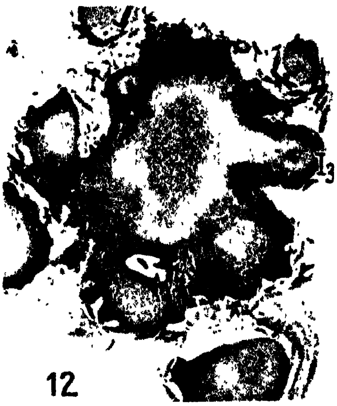
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C. W. WARDLAW

Two New Algae from Indian Soils[1]

BY

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(Department of Botany, Queen Mary College, London, and University of Allahabad, India)

With forty-five Figures in the Text

THE algae described in this paper appeared in cultures of desiccated soils collected from cultivated and uncultivated land near Allahabad, India. The new cultures were prepared with De's (1939, p. 124) modification of Benecke's solution. The algae involved were reared in unialgal cultures, mainly obtained by streaking on agar, followed by growth in De's solution with a little soil extract or on solid media prepared by adding 1·5 per cent. agar to the preceding.

1. *Chloranomala palmelloides* gen. et sp. nov. (Figs. 1–24)

The palmelloid colonies of this alga appear as a thin, light green, easily breakable film on the surface of the culture solution or on the sides of the vessel. The film consists of a hyaline mucilaginous matrix containing globular cells in all stages of division. The individual cell is surrounded by a special hyaline mucilage layer, which varies in thickness according to its degree of hydration and is easily seen after staining with ruthenium red (Fig. 1). The cells possess a light green parietal chloroplast, with lobed edges, not occupying the entire contour of the cell and containing a pyrenoid with a starch-sheath. Staining with iodine, ruthenium red, or Noland's reagent fails to reveal the presence of pseudo-cilia.

Before division the side of the cell away from the pyrenoid broadens so that the cytoplasm harbouring the nucleus becomes more obvious (Fig. 2). The pyrenoid divides by constriction. The daughter-cells are usually of equal size (Figs. 3, 4), although sometimes very unequal (Fig. 5). The first division is soon followed by a second in a plane at right angles to the first, the two daughter-cells of the first division dividing simultaneously or successively (Fig. 6). The four resulting cells slowly separate as mucilage is secreted and gradually acquire a rounded shape (Figs. 6–10).

When transferred to water the mucilage swells and the cells become widely separated. The protoplasts acquire flagella and, in 20–30 minutes, wriggle out as naked swarmers. Meanwhile a marked change takes place in the appearance of the cell-contents. The chloroplast becomes paler and obscured by an accumulation of large numbers of small oil-globules, with a bluish-green glint and situated chiefly in the neighbourhood of the chloroplast. The pyrenoid becomes difficult to recognize and, when visible, the starch-sheath

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[1] Part of a thesis presented for the Ph.D. degree of the University of London.

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appears much thinner or may even be absent (Figs. 9, 10, 13). During swarmer-formation most, if not all, of the starch seems to be converted into oil and treatment with iodine affords very little blue coloration.

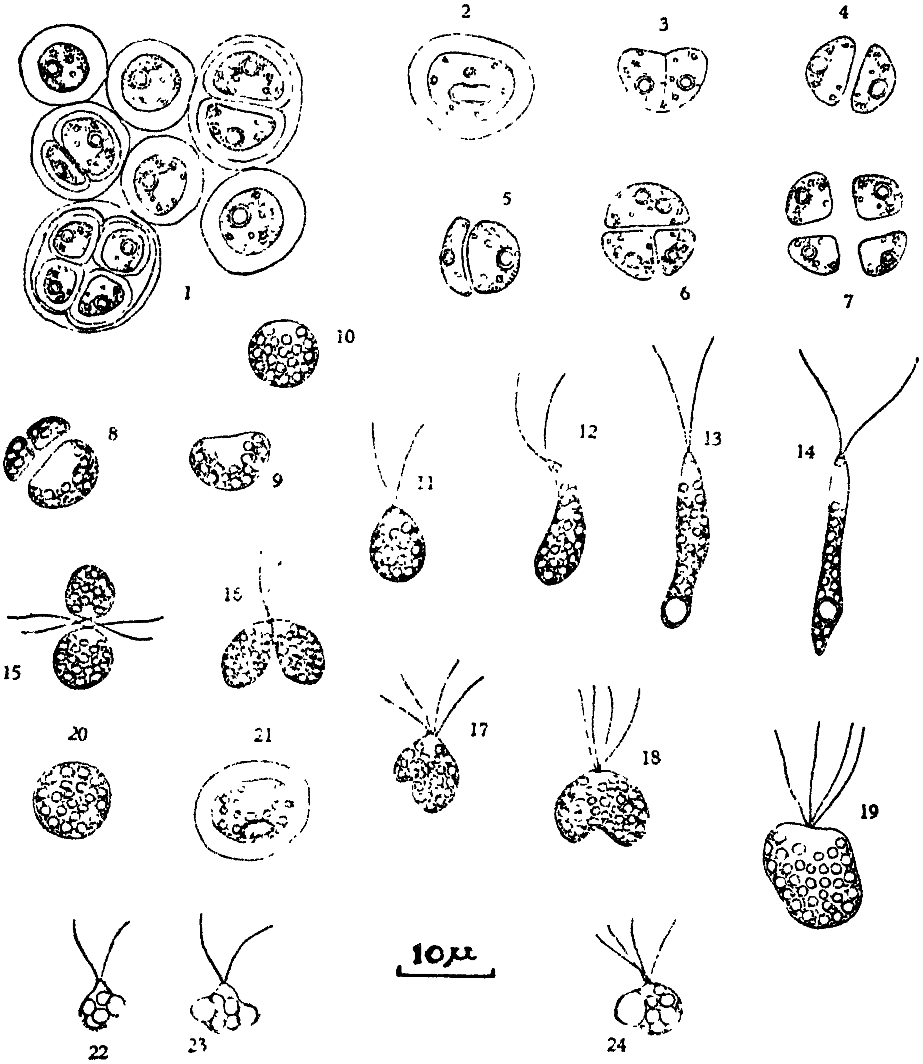
The swarmers (Figs. 11–14) possess two equal flagella, attached to the hyaline anterior end and about as long as the body. The swarmers are highly metabolic, at times being globular or ovoid and then measuring 3–5 μ in diameter, while at other times they may lengthen to about 20 μ. The thin cup-shaped chloroplast is obscured by the numerous oil-globules. The pyrenoid is rarely discernible, and is seen especially near the posterior end of the much elongated individuals (Figs. 13, 14), but its position is variable, owing to the metaboly of the cell. Two contractile vacuoles, pulsating alternately, lie in the clear cytoplasm at the anterior end. There is no eye-spot and the swarmers are almost indifferent to the direction of the incident light.

Except for a small anterior region, the swarmer is filled with the above-mentioned globules, which give all the tests for fatty oil. When the cells are crushed, the globules coalesce to form large drops which, like the globules within the swarmer, stain bright red with Scharlach red. With osmic acid they gradually blacken. When treated with dilute iodine the swarmers contract and the unstained globules coalesce into a few larger ones, some of which protrude from the cells (Figs. 22–4). The globules are completely dissolved by benzol and subsequent treatment with Scharlach red, after removal of the benzol, affords no coloration. The various tests have been repeatedly performed and suggest that the globules are fat.

The swarmers continue to move for 5–8 hours and finally come to rest on the surface or at the bottom of the liquid. They assume a spherical shape and soon secrete a mucilaginous envelope. After a considerable length of time the oil-drops diminish in number, and the chloroplast and pyrenoid again become prominent.

The swarmers may also behave as facultative gametes which fuse during movement. They aggregate in clumps, comprising 3–50 swarmers, all of which retain their motility. Although there is frequent conjugation between two equal gametes (Figs. 15, 16), a larger gamete is usually seen to be followed by two or three smaller ones, with one of which it fuses (Fig. 17). The gametes come together by their anterior ends, becoming entangled by their flagella (Fig. 15), and then coming to lie side by side (Fig. 16). Fusion commences at the anterior and progresses towards the posterior end (Figs. 17, 18). The process of pairing takes about 10–15 minutes, but fusion is completed in about a minute. The naked quadriflagellate zygote (Fig. 19) continues to move for several hours, ultimately, like the swarmers, rounding off and secreting a mucilage envelope (Figs. 20, 21). After a time division into four daughter-cells takes place. Although this organism has been cultivated in unialgal cultures on a variety of solid and liquid media for a considerable time, no resting-stages have ever been observed. On drying agar the envelope becomes firmer and most of the cells die. When placed in water the contents of the surviving cells emerge through a gelatinized part of the envelope.

The nature of the chloroplast, the production of starch, and the presence of two equal anterior flagella in the swarmers indicate that this alga should be classed among the Chlorophyceae. The palmelloid colonies, in fact, suggest a reference to the Palmellaceae, and the cell-structure would be in agreement with this. The copious formation of oil-globules, which precedes the liberation of swarmers and characterizes the motile stages, is a unique feature that



FIGS. 1–24. *Chloranomala palmelloides* gen. et sp. nov. Fig. 1. Cells embedded in mucilage. Figs. 2–7. Stages in cell-division. Figs. 8–10. Appearance of oil-globules in incipient swarmers. Figs. 11–14. Swarmers. Figs. 15–18. Stages in sexual fusion. Fig. 19. Zygote. Figs. 20–1. Vegetative cells derived from zygotes showing secretion of mucilage. Figs. 22–4. Swarmers and zygote stained with iodine, showing coalescence of oil-globules.

renders it impossible to refer this alga to any known genus of Green Algae. The possibility of a parasite being involved was examined critically. Unialgal cultures were prepared several times by streaking on agar. Single swarmers were isolated and observed throughout their development, but no evidence of the presence of a parasite was ever obtained.

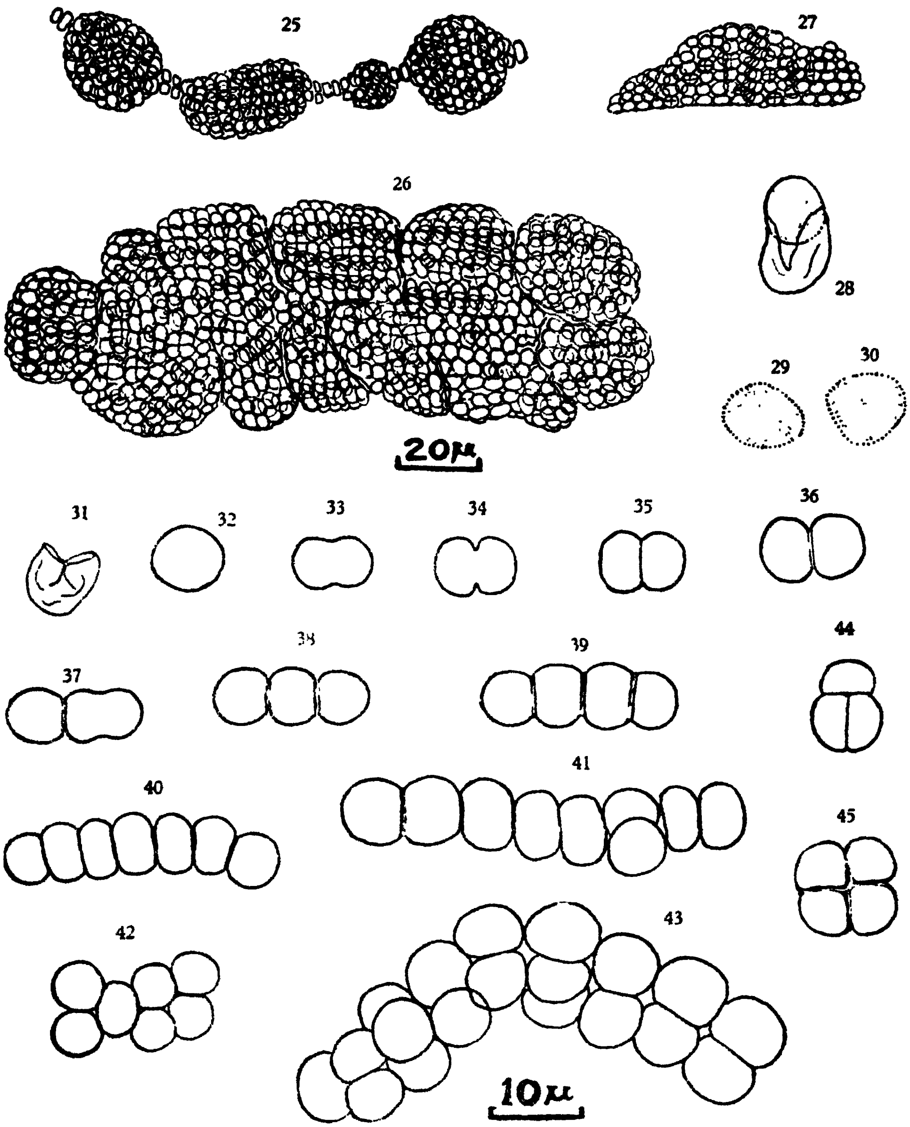
The method of sexual reproduction resembles to a certain extent that described for *Tetraspora lubrica* by Geitler (1931, p. 177), but there are important differences in the structure of the gametes and in their metaboly and physiological behaviour. Moreover, as no evidence of the presence of pseudocilia has been obtained, an affinity with Tetrasporaceae is doubtful. The alga may, therefore, provisionally be referred to the Palmellaceae.

2. *Chlorogloea Fritschii* n. sp. (Figs. 25–45)

This alga formed a deep blue-green amorphous crust, about 500 μ thick, on the sides of the culture vessel, as well as on soil, and a thinner, though more expanded, stratum on the surface of the liquid. These growths consist of large, loosely and irregularly superposed, packets which are visible to the naked eye and consist of rounded chroococcoid cells (Fig. 26) of variable size. No mucilage-envelope is recognizable around the individual packets, even after staining with ruthenium red or mounting in indian ink, although the cells cohere firmly. The cells themselves are also without any evident enveloping mucilage, although the cell-wall stains with ruthenium red, methylene blue, or cotton blue. In the young condition the cells are arranged in longitudinal and transverse rows (Fig. 43), but this arrangement is later obscured by displacement during division and enlargement. Although generally rounded, the cells may become polygonal by mutual pressure. In older cultures the membranes of the peripheral cells become pale brown and, after discharge of the contents, the empty walls always show a distinct brownish tinge. The cell contents are pale blue-green and contain a few large scattered granules.

Cell division takes place by gradual constriction along three planes at right angles to one another. As a general rule the cells enlarge and round off after division, but groups of four cells are often observed. Within the individual packets the cells, in optical section (Fig. 27), appear arranged in vertical and horizontal rows.

Any cell can produce a single endospore, liberated by rupture of the envelope (Fig. 28) which subsequently collapses and becomes folded (Fig. 31). The endospores are naked, devoid of a mucilage-envelope, and correspond in shape to that of the parent cell. They are usually globular, but sometimes a little flattened on one side (Figs. 29, 30). The contents are pale blue-green and finely granular. Sometimes the endospores undergo constriction before liberation and in rare instances may even exhibit division into two. These are probably examples of premature germination, since the cells remain joined after liberation and do not constitute separate endospores. Sometimes an ordinary cell of a packet divides into a number of small ones, but each such cell has an envelope of its own and can liberate an endospore.



Figs. 25–43. *Chlorogloea Fritschii* n. sp. Fig. 25. Part of a growth from a liquid culture containing ammonium salts. Fig. 26. Usual growth, showing packets of cells. Fig. 27. Section of a packet. Fig. 28. Liberation of endospore. Figs. 29–30. Naked endospores. Fig. 31. Empty membrane after liberation of endospore. Figs. 32–40. Stages in the germination of endospores, showing formation of a uniseriate filament. Figs. 41–3. Formation of filaments with two or three rows of cells. Figs. 44–5. Division of detached cells.

The naked endospores subsequently always assume a globular shape and secrete a delicate envelope. After slight elongation they divide into two by constriction (Figs. 33–6). Further divisions in the same plane (Fig. 37) give rise to a short, straight or curved, uniseriate filament of 3–12 cells which is clearly constricted at the septa (Figs. 38–40) and without any mucilage-envelope. The outer surface of the end-cells is broadly rounded, and these cells are sometimes larger or smaller than the others. Subsequently certain cells of the filament begin to divide along a plane at right angles to that of the earlier ones (Fig. 41), and by degrees other cells follow suit. These divisions lead to the formation of a biseriate filament (Fig. 42), but this stage is transitory, since divisions soon take place along three planes at right angles, so that the filamentous character becomes obscured (Fig. 43). Since every cell of the primary filament divides in the same way and similar divisions take place in the daughter-cells, the cells of the resulting packets are arranged in vertical and transverse rows. Endospores often germinate near their place of liberation and may form packets overlying that from which the spore was produced.

Another method of reproduction consists in the separation of single cells or of groups of two or three cells from the packets. These develop into short filaments in the same way as the endospores, but division in all three planes usually sets in early.

In liquid cultures, and especially in media containing ammonium nitrate, only some of the cells of the primary filaments continue to divide and form packets, while others, with or without division, begin to degenerate. This gives the resulting growth a jointed appearance (Fig. 25), with irregular packets of blue-green cells strung together by strips in which the degenerating cells appear yellowish. This rarely happens in agar cultures.

The mature growths of this alga resemble a Microcystis, except for the orderly arrangement of the cells in rows and the frequent grouping of the packets in linear series. This latter feature suggests a reference to the Entophysalidaceae (Fritsch, 1945, p. 818). There are some resemblances to *Chlorogloea microcystoides* Geitler (1925, p. 357; 1932, p. 310), the only freshwater species so far recorded, in which, however, the early development is unknown. The Allahabad alga may be provisionally referred to Chlorogloea, pending an investigation of the early stages of *C. microcystoides*, though the differences may prove to be great enough to warrant the establishment of a separate genus.

The general shape of the growths and the disposition of the cells in rows constitute the chief resemblances to *C. microcystoides*, but the habitat is different and there is no common mucilage-envelope around the packets; the cells are also larger and more loosely disposed. When viewed in optical section the rows of cells afford no evidence of the branching seen in Geitler's species. The most important difference, however, lies in the method of reproduction. In *C. microcystoides* a number of endospores are formed within enlarged cells, while in *C. Fritschii* the endospores are produced singly within ordinary cells. The formation of a primary filament has not been recorded among the Entophysalidaceae, although it occurs in diverse Pleurocapsales, such as *Pleurocapsa minor* Geitler (1925, p. 343). Here, however, the terminal cell of the filament behaves as an apical cell and shows oblique division.

Chlorogloea Fritschii also shows some resemblance to *Chroococcopsis* *fluminensis* Fritsch (1929, p. 183), which Geitler (1932, p. 323) would include in *Pleurocapsa minor* owing to occasional production of upright rows of cells. *Chroococcopsis fluminensis* forms a similar deep blue-green stratum consisting of loose aggregates of chroococcoid cells giving rise to single endospores, whose germination is unknown.

If *Chlorogloea Fritschii* is rightly referred to the Entophysalidaceae, it affords a link between this family and the Pleurocapsaceae. Fritsch (1942, p. 138; 1945, p. 858) doubts the derivation of Pleurocapsales from the Entophysalidaceae, advocated by Geitler (1932, p. 94 and p. 293), since there has hitherto been no evidence of the occurrence of a juvenile filament in the Entophysalidaceae. The presence of such a stage in *C. Fritschii*, however, appears to lend support to such a derivation.

DIAGNOSIS OF THE NEW FORMS

Chloranomala palmelloides gen. et sp. nov.

Cells globular or hemispherical after division, 4–8 μ in diameter, embedded in soft mucilage to form a thin film; chloroplast parietal, cup-shaped with lobed edges, light green, the pyrenoid usually opposite the opening of the chloroplast. Multiplication by division of cells along two planes at right angles to one another, the daughter-cells provided with individual mucilage-envelopes. Swarmers with two equal flagella strongly metabolic, ovoid to elongate, 3–5 μ in diameter and up to 20 μ long, the parietal chloroplast with a basal pyrenoid obscured by numerous oil-globules, contractile vacuoles two, anterior; no eye-spot. Swarmers can behave as gametes, showing iso- or anisogamous fusion after clump-formation; zygote 4-flagellate, motile, like the non-conjugating swarmers rounding off after some time and dividing to form a new palmelloid colony.

Hab. In cultures of desiccated soils from rice-fields and gardens, as well as from usar and red soils, India.

Chlorogloea Fritschii n. sp.

Stratum a deep blue-green crust of indefinite size, composed of rounded or irregular cell-packets. Cells arranged in vertical and horizontal rows, rounded or angular, without evident mucilage-envelopes, with pale blue-green granular contents, diameter 4–12 μ (usually 6–8 μ). Reproduction by (1) naked spherical endospores, 4–9 μ broad, formed singly within the cells and liberated by rupture of the membrane, and (2) separation of single cells or groups of two or three cells. Endospores give rise to a short prostrate uniseriate filament of 3–12 cells which divide in three directions to produce the packets.

Hab. In cultures of cultivated and garden soils from Allahabad, Northern India.

SUMMARY

A new monotypic genus *Chloranomala palmelloides*, provisionally referred to the Palmellaceae, is described. The highly metabolic motile cells contain

large quantities of oil globules and either germinate without fusion or behave as gametes.

A new species of *Chlorogloea* (*C. Fritschii*), whose endospores germinate to produce an at first uniseriate filament, is described.

The author is indebted to Professor F. E. Fritsch, F.R.S., for advice and guidance during this investigation.

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Growth Analysis of the Sugar-cane Crop in North Bihar (India)

I. Seasonal Variation in Growth and Yield in Unmanured Plots

BY

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With five Figures in the Text

INTRODUCTION

IN North Bihar in north-east India is located the second largest white-sugar belt in India. This tract is situated in the sub-tropical Indo-gangetic plain and is perhaps the only one of its kind in the world where sugar-cane as a commercial crop is grown without irrigation.

The climate of North Bihar has a fairly cold winter with sometimes a little rain at intervals. Frost is very rare. From March to June the weather is hot and the maximum temperature occasionally rises above 100° F. There are often storms towards the end of the hot weather and frequently useful rain. The monsoon usually breaks by the middle of June and from then to the middle of September an average rainfall of 45 in. (110 cm.). Vegetative growth of sugar-cane practically ceases by the middle of October.

The soil varies from light sandy loam to medium silty loam. There is a high proportion of lime. The soil is interspersed with beds of sand through which water seeps sideways underground from the rivers. It is this combination of a comparatively mild climate with the fertile moisture-holding and supplying soil that makes cane-growing without irrigation possible.

Sugar-cane is grown as an annual crop in North Bihar, and the average yield is perhaps the lowest in India, approximating to about 15 tons per acre. The Coimbatore canes are grown over almost the whole area. The problem of increasing tonnage per acre is very pressing. Manuring with nitrogenous and phosphatic fertilizers has, however, been found to have an inconsistent effect. It was therefore considered worth while to undertake a physiological analysis of growth under field conditions on the lines formulated originally by Gregory (1917) and Briggs, Kidd, and West (1920). Similar studies undertaken in the case of cotton by Crowther (1934, 1941) in the Sudan and Heath (1937) in East Africa and in the case of beetroot and cereals by Watson (1938, 1947) in England have yielded valuable results. Observations reported in this paper were made at Pusa, where conditions are representative. The effects of nitrogen, phosphorus, and irrigation on growth and yield were investigated during

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four seasons. Observations on unmanured plots alone are reported here. The variety used, Co 313, is very widely cultivated in this region.

A brief reference to the usual agronomic practice may not be out of place. The second half of February appears to be the most suitable time for large-scale planting, as the temperature then is not too low and soil moisture is just enough for germination to commence. With the progressive delay in the date of planting the soil moisture tends to diminish with rising temperature and decreasing relative humidity. Cane sets are planted end to end in a furrow opened by a ridge-plough. The furrow is then closed by a 4-tine Bihar cultivator and the field is then levelled off by beaming. Immediately after the monsoon has set and before the cane has grown too tall to get the bullocks through it without damage the crop is earthed up by driving a ridge-plough through the rows. The ridge-plough throws the earth up into ridges along the rows of the cane and leaves furrows between the rows. A 3-year rotation is the usual practice. In the preceding summer the land is kept either fallow or put under early paddy, maize, or a green manuring crop. At the time of preparatory tillage in early December, farmyard manure at the rate of $3\frac{1}{2}$ tons per acre is applied if the land has been under paddy or maize.

EXPERIMENTAL DETAILS

The data under discussion relate to observations on control plots from field experiments with all combinations of three levels of nitrogen and phosphoric acid applied in the form of castor cake and double superphosphate respectively at the time of planting. The treatments were replicated three times and also randomized. Plot size was 60·5 ft. by 30 ft.—$\frac{1}{24}$ acre—and included ten rows 3 ft. apart. The central four rows ($\frac{1}{60}$ acre) were reserved for final yields and periodic observations on height, tillering, &c.; plant samples were collected from the other four rows, two on each side of the four central rows. The remaining two rows on each end of the plot served as border rows. The seed rate was constant throughout—64 healthy 3-bud sets per row (60·5 ft. long).

The first sample was collected about 8 weeks after planting when germination was complete and just before the appearance above ground of the first tiller. Samples were collected regularly at intervals of 4 weeks until the shoot ceased elongating. Thus about seven samples were collected during the growing period. Four clumps (aerial portion cut at ground level) were collected at random from each plot, one from each row. With three replications a complete sample per treatment consisted of twelve clumps. The size of the sample was fixed according to convenience and facilities available in the laboratory. Each row was divided into seven equal lengths, to each of which was assigned a number ranging from 1 to 7. From one of these numbered lengths the corresponding sample was collected, which consisted of a clump of average size (as judged by eye). Statistically randomized sampling had to be thus compromised because the sugar-cane crop is never very uniform, the time taken for germination (which was at most about 33 per cent.) extending over weeks during which the seedlings are also ravaged by pests.

In the laboratory the plant samples were separated into fully unfolded green leaves, sheaths, stems, and dry leaves. The partially unfolded green leaves were mixed with sheaths. After cutting the various parts into small bits of convenient size the whole sample was quickly weighed and a small sub-sample amounting to about 10 to 20 per cent. of the original weight was again weighed out and dried. Drying was effected in large wooden cabinets specially constructed for the purpose. There was no bottom to the cabinet, and the top could be slid open for letting out moisture-laden air. In the bottom was placed an electric heater over which rested a large tin sheet. The shelves were made from drawn metal. The sample trays were made from bamboo strippings. The upper shelves did not receive the same degree of heat as the lower ones and therefore all the samples were dried on the two lower shelves for the same length of time (at about 70° C.) before weighing. The dried samples were powdered in a hand-mill, sieved, and stored in sealed containers for chemical analysis later.

It was considered necessary to make some adjustment in the dry weights of the samples. Attacks by borers caused irregular damage to the shoots (dead hearts) and it was difficult to pick out from within an 8·5 ft. length a clump of average size. This difficulty was experienced particularly after the internodes elongated and cane formation started. The dry weight of the samples collected after this stage were, therefore, corrected on the basis of mean number of healthy canes per clump per plot (obtained from periodic shoot counts of the four central rows).

Shoots in the four central rows (reserved for yield) were periodically counted and the number of dead hearts was also recorded. Three shoots in each row were tagged at random and heights of twelve such shoots (from a fixed point at ground level to the transverse mark on the topmost fully expanded leaf) were also recorded at periodical intervals. Observations on the production and death of leaves were also recorded. If a shoot was affected by dead heart, it was substituted by another healthy shoot.

METHODS OF GROWTH ANALYSIS

From the sampling data of dry weights estimates of net assimilation rate were obtained by methods used by Gregory (1926). The net assimilation rate was calculated on the basis of unit dry-weight of green leaf instead of unit area owing to the difficulty of estimating leaf-area in the field. From the dry-weight data and the nitrogen content the uptake of nitrogen was calculated as absolute rate of uptake. (Total nitrogen was estimated by the usual Kjeldahl method after reducing nitrate in the cold by the salicylic acid sulphuric acid mixture.) The importance of estimating the uptake of phosphorus and potassium was recognized, but lack of adequate laboratory facilities precluded their analysis.

The mean rates of production and mortality of tillers were calculated thus: if at times t_1 and t_2 the number of all shoots be N_1 and N_2 and the number of 'dead hearts' D_1 and D_2 respectively, then the rate of production between interval t_1 to t_2 is $[(N_2-N_1)/N_1]\times 100$ and the rate of mortality is

$$[2(D_2-D_1)/(N_1-N_2-2D_1)]\times 100.$$

FINAL YIELDS

In Table I are given the yields of millable cane along with other relevant particulars. It will be seen that there is an appreciable variation in yield from year to year. Difference in germination or cropping during the preceding summer does not correlate with variation in yield. Discussion of the causes of differences in yield would appear to be facilitated by comparing growth data of each experiment with those of Bhograsan (which gave the highest yield) taken as the standard. Only those data which bear directly on the points under consideration have been presented.

TABLE I

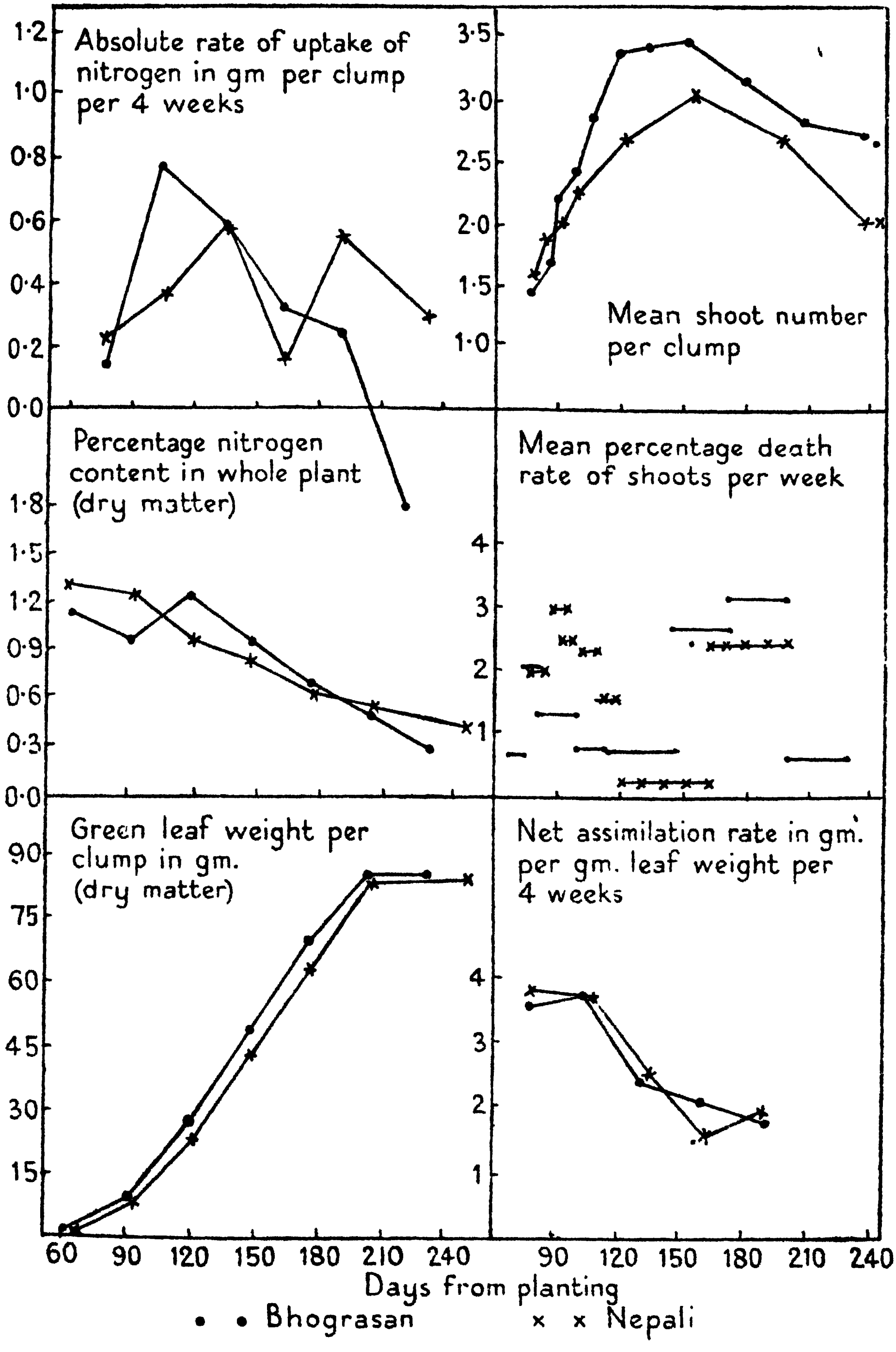
Field.	Soil type.	Preceding summer crop.	Mean yield (tons/acre).	Planting date.	Germination %.
North Pangarbi	Sandy loam	Green manure	12·96	14/2/42	30·0
Brickfield	Silt loam	Fallow	12·96	5/3/45	32·7
Nepali	Sandy loam	Fallow	15·80	24/2/43	32·0
Harpur Jhilli	Silt loam	Fallow	16·19	8/3/43	34·0
Chonia	Silt loam	Maize	19·02	3/3/44	26·0
Bhograsan	Sandy loam	Fallow	21·78	23/2/44	32·0

DEVELOPMENTAL OBSERVATIONS AND SAMPLING DATA

Bhograsan and Nepali

The course of tillering is indicated (Fig. 1) as mean number of living shoots per clump plotted against time in number of days from planting. The tiller production in Nepali was identical with, or even slightly superior to, that in Bhograsan in the early stage, but lagged behind after about 13 weeks. The maximum difference between the two was reached by about 17 weeks. Subsequently the difference diminished, but the difference as regards the mean number of mature canes per clump at harvest was almost as wide as that between tiller numbers at 17 weeks. Evidently the tillers in Nepali that developed later did not mature into millable canes. It may also be noted that the mean percentage death-rate was consistently higher in Nepali up to about 17 weeks. This does not, however, account for the difference in tiller number by the end of the period because the mean rate of production was substantially higher in Bhograsan. It may be noted that the maximum on the tiller curve in Nepali was attained later and was also at a lower level than that in Bhograsan.

Maximum green-leaf weight was attained in both by 29 weeks. Difference in leaf weight between the two was apparent by 13 weeks; it widened gradually up to about 25 weeks and then diminished. Since meristematic activity as measured by tillering and leaf production is dependent to a large extent upon nitrogen supply, the relation between nitrogen uptake and tillering and leaf growth may now be examined.



Absolute rate of uptake of nitrogen in gm per clump per 4 weeks
1·2
1·0
0·8
0·6
0·4
0·2
0·0
Mean shoot number per clump
3·5
3·0
2·5
2·0
1·5
1·0
Percentage nitrogen content in whole plant (dry matter)
1·8
1·5
1·2
0·9
0·6
0·3
0·0
Mean percentage death rate of shoots per week
4
3
2
1
Green leaf weight per clump in gm. (dry matter)
90
75
60
45
30
15
Net assimilation rate in gm. per gm. leaf weight per 4 weeks
4
3
2
1
60
90
120
150
180
210
240
90
120
150
180
210
240
Days from planting
• • Bhograsan
× × Nepali

FIG. 1.

The absolute rate of uptake of nitrogen (Fig. 1) in Bhograsan reached maximum level by 15 weeks, then gradually diminished and fell to zero by 30 weeks. In Nepali the rate of uptake of nitrogen rose to maximum (though to a lower level as compared to Bhograsan) by about 19 weeks, then diminished gradually until about 23 weeks, and then again rose to a secondary maximum. Up to the first 13 weeks the rate of uptake of nitrogen was more or less identical in both Nepali and Bhograsan, which correlated with more or less similar tillering rate and leaf growth in both. Subsequently the slopes of the respective curves of uptake of nitrogen diverged considerably and also those of the tillering and leaf-weight curves. The maximum difference in tillering at 17 weeks was apparently conditioned by maximum difference in uptake rate between 13 and 17 weeks.

During the first 13 weeks the nitrogen content was higher in Nepali. During the succeeding 12 weeks—13th to 25th—the rate of uptake of nitrogen as well as percentage nitrogen content was on the whole higher in Bhograsan, which may be interpreted as indicating greater availability of nitrogen in Bhograsan during this period and also that nitrogen limited growth in Nepali. After 25 weeks nitrogen appeared to be increasingly available in Nepali, as indicated by the rise in the rate of uptake as well as increase in the rate of leaf growth, with the result that final leaf weight was almost as high as that in Bhograsan. The final yield was, however, lower in Nepali because, although the crop made up in growth at a later stage, this growth was confined to individual stalks and not to increase in their number, the tillering phase having reached its peak earlier. It may be noted that the net assimilation rate was more or less identical in the two cases and thus in no way affected growth in Nepali.

The weather data given in Table II indicate that there was no heavy rain between the 13th and the 25th week to account for washing down of nitrate below root depth when nitrogen appeared to be limiting growth in Nepali. With the data at hand it cannot be conjectured how nitrogen became deficient between 13 and 25 weeks and relatively more available subsequently. In any case the growth analysis indicated the operation of some adverse soil factor in Nepali which was situated only a few yards away from Bhograsan. Bhograsan is also noted at Pusa for its fertility.

TABLE II

Weather Data

Days from planting.		Rainfall in inches.		Mean temperature in °F. Maximum.		Mean temperature in °F. Minimum.	
		N*	B*	N*	B*	N*	B*
0–91	Nitrogen not limiting	2·80	1·42	92·9	92·6	67·7	66·6
91–105	Nitrogen limiting	1·75	2·09	97·6	98·5	78·6	77·5
105–75	Nitrogen limiting	17·71	18·12	91·7	92·7	78·8	79·4
175–210	Nitrogen available	8·84	8·22	89·7	91·2	78·9	78·3

* N for Nepali; B for Bhograsan.

Bhograsan and Harpur Jhilli

Harpur Jhilli was ahead of Bhograsan as regards tillering for the first 13 weeks. The Harpur Jhilli crop was planted earlier by about 2 weeks and it is possible that it thus got a better start due to milder weather during this period. After this time the rate of tillering in Harpur Jhilli decreased rather suddenly. It may be noted, however, that a day before the next shoot-count was taken the crop was earthed up and, therefore, very likely a large number of young tillers were buried and could not be counted. Assuming that the same steady rate of increase continued, the mean number of shoots per clump would have been about three by the 15th week and therefore still above that in Bhograsan. Soon after, however, tillering in Bhograsan surpassed that in Harpur Jhilli, and a sharp break in the curve of tillering in the former occurred approximately by the 17th week. The crop was earthed up 3 days after this shoot-count, and there was only a slight increase in tillering after this time. After the 15th week, when tillering in Bhograsan overtook and surpassed that in Harpur Jhilli, it may be noted that the latter did not suffer from higher death-rate. In fact the death-rate was slightly greater in Bhograsan, but the production rate was much higher. The difference in mature canes at harvest was practically the same as that for the maximum tiller number.

Maximum green-leaf weight was attained in Bhograsan by 29 weeks and in Harpur Jhilli by 25 weeks. Leaf weight was, however, higher in Harpur Jhilli from the beginning until about 21 weeks. After this time it increased very little in Harpur Jhilli, but at a considerable rate in Bhograsan, with the result that the maximum leaf weight was higher in the latter.

For the first 13 weeks the rate of uptake of nitrogen as well as the percentage nitrogen content was higher in Harpur Jhilli. Tillering as well as leaf growth was maintained at a higher level. Between 13 and 17 weeks the rate of uptake of nitrogen was only slightly greater in Harpur Jhilli, whereas the percentage content decreased and almost equalized with that in Bhograsan. It would seem that soil nitrogen supply had become relatively deficient in Harpur Jhilli during this period. The falling off in tillering after the 15th week in Harpur Jhilli may also be noted in this connexion. After 17 weeks the rate of uptake was consistently lower in Harpur Jhilli and the uptake also ceased altogether much earlier, which accounts for the much earlier cessation of leaf growth.

It may be considered why soil nitrogen in Harpur Jhilli became relatively in short supply from the 13th week onwards. Harpur Jhilli being situated in a low-lying area, the soil got quickly saturated with moisture after the onset of the monsoon and remained so throughout the rainy season and even some time later. A green scum of algal growth could also be seen on the surface throughout the rainy season. It was at times difficult to walk between the rows for observation purposes on account of the slush, and hence the paucity of observations on tillering after the 15th week. Data on rainfall given in Table III indicate that from the 10th week onward there was a substantially heavier rainfall in the case of Harpur Jhilli which might have washed down soil nitrate. Alternatively, due to the excessive accumulation of soil moisture,

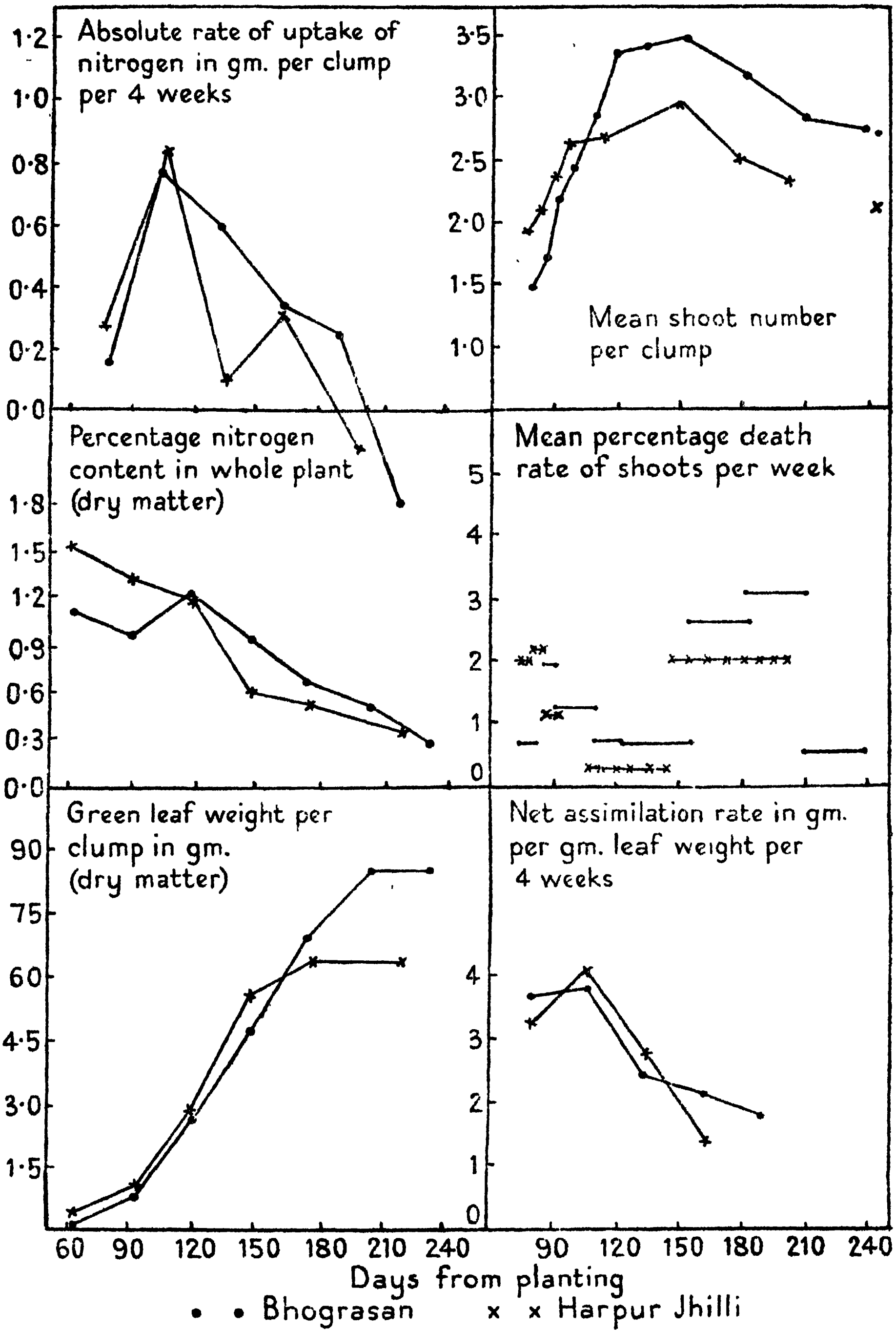

Absolute rate of uptake of nitrogen in gm. per clump per 4 weeks
1·2
1·0
0·8
0·6
0·4
0·2
0·0
Mean shoot number per clump
3·5
3·0
2·5
2·0
1·5
1·0
Percentage nitrogen content in whole plant (dry matter)
1·8
1·5
1·2
0·9
0·6
0·3
0·0
Mean percentage death rate of shoots per week
5
4
3
2
1
0
Green leaf weight per clump in gm. (dry matter)
90
75
60
4·5
3·0
1·5
Net assimilation rate in gm. per gm. leaf weight per 4 weeks
4
3
2
1
0
60
90
120
150
180
210
240
90
120
150
180
210
240
Days from planting
• • Bhograsan
x x Harpur Jhilli

FIG. 2.

nitrification or absorption capacity of roots might have been inhibited. It is also possible that all these factors might have operated together or singly during this period. Be that as it may, it is clear that due to some soil condition comparatively less nitrogen was available to the Harpur Jhilli crop from the 13th week onwards.

TABLE III

Rainfall in Inches

	Time in weeks from planting				
	9th	10th	13th	14th	15th
Harpur Jhilli . . .	0·13	1·07	0·70	2·05	1·70
Bhograsan . . .	0·13	0·07	0·00	0·00	2·09

The other growth data are compared in Table IV. It will be seen that shoot growth in Harpur Jhilli was more rapid in the early stages. Cane formation also commenced earlier, as indicated by the proportion of stem weight in the whole plant. This earlier start in growth may be ascribed to better nitrogen and moisture status of the soil. Subsequently accumulation of moisture limited nitrogen supply and slowed down tillering, but did not apparently affect extension phase of growth of the parts already differentiated. It may also be noted that the net assimilation rate in Harpur Jhilli was relatively low between 21 and 25 weeks. Thus by 25 weeks practically the same amount of dry matter accumulated in both cases, as illustrated by the data in Table V, although its distribution among various parts of the plant was different.

TABLE IV

Growth Data, Heights of Plants (in inches)

Harpur Jhilli				Bhograsan			
Days after planting.	Plant height* (in.).	Stem height† (in.).	$\frac{\text{Stem weight}}{\text{Total weight}} \times 100$	Days after planting.	Plant height* (in.).	Stem height† (in.).	$\frac{\text{Stem weight}}{\text{Total weight}} \times 100$
64	7·8	—	—	63	7·0	—	—
92	12·5	—	—	91	10·4	—	—
120	24·7	—	—	119	18·9	—	—
148	—	23·7	38·0	147	32·9	14·4	29·0
176	—	40·3	50·0	175	—	27·8	43·0
				203	—	45·7	50·0
218	—	57·0	60·2	231	—	59·8	58·0

* Main shoot only. † All canes in a clump.

TABLE V

Accumulation of Nitrogen and Dry Weight

	Harpur Jhilli. (g.)	Bhograsan. (g.)
Total nitrogen content per clump	1·62	1·93
Total dry weight	306·6	292·9
Green leaf weight	67·8	70·0
Stem weight	153·4	126·2

After 25 weeks little leaf growth was made in Harpur Jhilli and a substantial difference in growth was established in the two fields after this time. Thus the Bhograsan crop outyielded the Harpur Jhilli crop mainly by virtue of its higher tiller number. It may be concluded that tillering in Harpur Jhilli was mainly depressed by nitrogen deficiency and the subsequent growth was checked by nitrogen shortage as well as other adverse conditions in the soil brought about by saturation with moisture.

Bhograsan and Brickfield

Although in the early stages the rate of tiller production was slightly higher in Brickfield (Fig. 3) the tiller number was practically equalized in the two cases by the 13th week. From this time until the 17th week tiller number was slightly higher in Bhograsan. In Brickfield tiller number actually decreased between 17 and 22 weeks due to lower production rate as well as a slightly higher death-rate. Between 22 and 26 weeks mortality was considerably greater in Bhograsan, so that by the end of the period the mean number of living shoots per clump was about the same in both. In spite of this the mean number of canes per clump at harvest was, however, significantly greater in Bhograsan. That this difference was not due to greater mortality in Brickfield can be seen from Table VI. Evidently cane formation was delayed in Brickfield.

TABLE VI

Numbers of Living and Dead Shoots

Brickfield				Bhograsan			
Date.	Total number of shoots per $\frac{1}{20}$ acre.	Dead hearts.	Living shoots.	Date.	Total number of shoots per $\frac{1}{20}$ acre.	Dead hearts.	Living shoots.
4/10	2,599	558	2,041	17/10	3,021	912	2,109
27/10	(Mature canes)	—	1,465	17/10	(Mature canes)	—	2,066
21/12	(,,)	—	1,524	4/12	(,,)	—	2,071

Maximum weight of green leaf was attained in both at about the same time, although the maximum levels were very different (Fig. 3). By about 13 weeks leaf weight in Bhograsan showed an increase over Brickfield and the difference widened further until about 20 weeks. Between 20 and 24 weeks the rate of leaf growth in Brickfield was higher than in Bhograsan, but subsequently it fell behind considerably in the former.

The absolute rate of uptake of nitrogen was practically indentical in both for the first 13 weeks or so, although the percentage nitrogen content was appreciably higher in Brickfield. Between 13 and 17 weeks, when the percentage nitrogen content was still higher, the uptake rate was lower in Brickfield. Evidently growth in Brickfield was restricted by some factor other that nitrogen and therefore nitrogen accumulated. Between 17 and 20 weeks growth in Brickfield diminished still further as compared to Bhograsan as indicated by the smaller increase in leaf weight and decrease in tiller number.

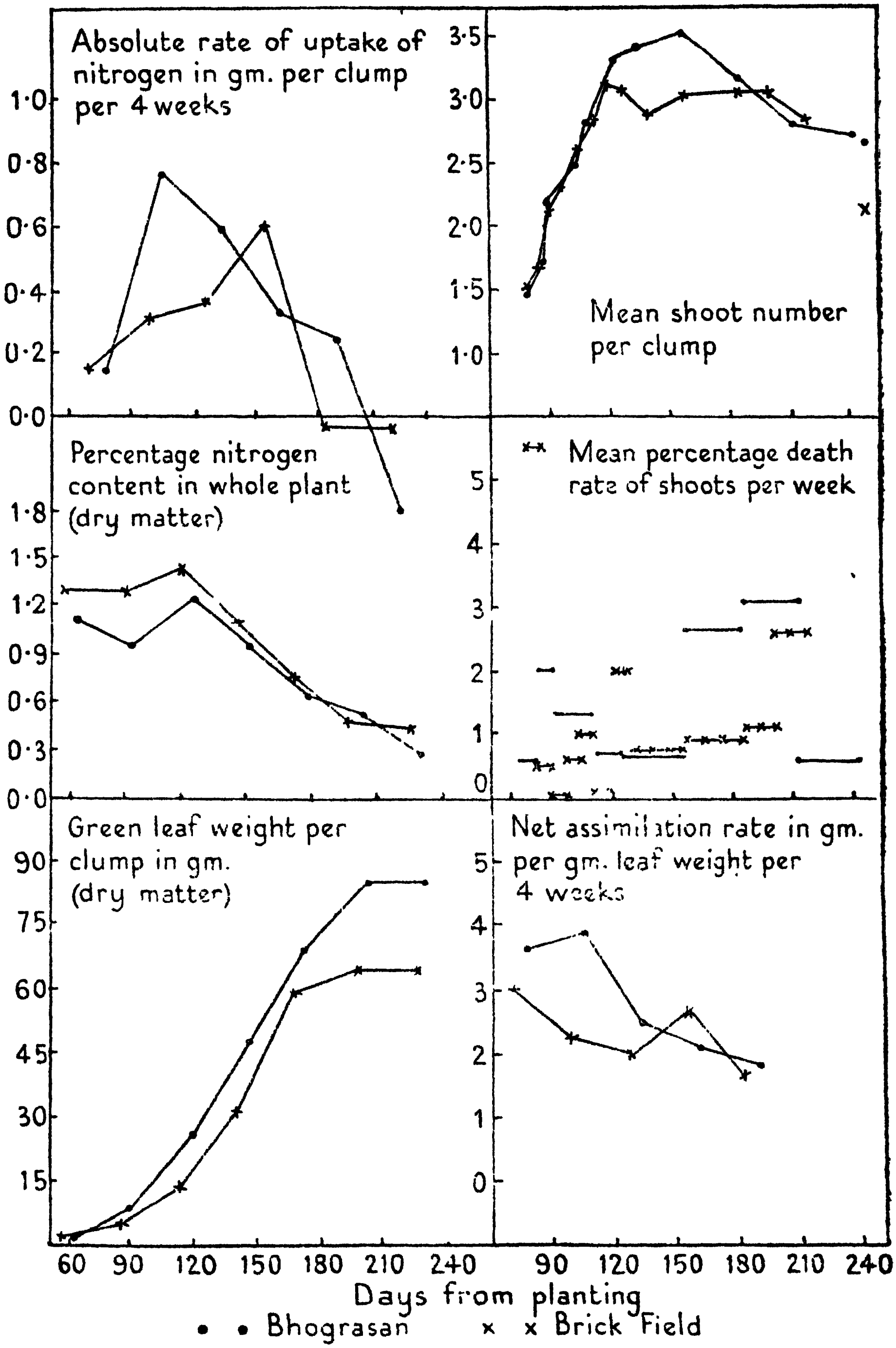

Absolute rate of uptake of nitrogen in gm. per clump per 4 weeks
1·0
0·8
0·6
0·4
0·2
0·0
3·5
3·0
2·5
2·0
1·5
1·0
Mean shoot number per clump
Percentage nitrogen content in whole plant (dry matter)
1·8
1·5
1·2
0·9
0·6
0·3
0·0
Mean percentage death rate of shoots per week
5
4
3
2
1
0
Green leaf weight per clump in gm. (dry matter)
90
75
60
45
30
15
Net assimilation rate in gm. per gm. leaf weight per 4 weeks
5
4
3
2
1
0
60
90
120
150
180
210
240
90
120
150
180
210
240
Days from planting
• • Bhograsan
× × Brick Field

FIG. 3.

During this period the uptake of nitrogen was also lower and the percentage nitrogen content higher in Brickfield, which again indicates that some factor other than nitrogen limited growth. Between 20 and 25 weeks growth improved in Brickfield, the rate of uptake of nitrogen also increased, and the percentage nitrogen content was about the same as in Bhograsan or slightly higher; this indicates that the factor that limited growth was no longer operative. From the 25th week onwards leaf growth in Brickfield was very poor as compared to Bhograsan, and this appears to be related to the much earlier cessation of nitrogen uptake.

From Fig. 3 it will be seen that the net assimilation rate was, on the whole, lower in Brickfield up to about 20 weeks.

From the data on rainfall given in Table VII it will be seen that March, April, and the first half of May during 1945 were dry as compared to 1944. During this period growth, however, did not suffer in Brickfield, probably because the soil moisture was just sufficient for the early stages of the crop. As already pointed out, growth in Brickfield lagged behind that in Bhograsan from the 13th week onwards until the 20th week, and this would seem to be accounted for by low rainfall during this period in 1945. Data in Table VIII indicate that soil moisture did not increase during July 1945 as it usually does after the hot weather, and it is conceivable that growth in Brickfield was checked between 13 and 20 weeks by the comparatively droughty conditions.

TABLE VII

Rainfall Data in Inches per Month

	March.	April.	May.	June.	July.
Rainfall in 1944 . .	0·86	0·54	2·09	5·44	7·01
Rainfall in 1945 . .	0·00	0·00	1·68	4·37	3·80

TABLE VIII

Percentage Moisture Content of Soil

	Brickfield		Bhograsan	
Date	1/6/45	25/7/45	11/5/44	6/7/44
	11·1%	11·1%	8·0%	15·6%

From the data in Table IX it will be seen that the effect of partial drought was also reflected in the moisture content of the stem, particularly in the July sample.

TABLE IX

Moisture Content of Stem as Percentage of Dry Weight

Date.	Bhograsan.	Date.	Brickfield.
26/5	291	28/5	398
21/6	527	25/6	483
19/7	600	22/7	406
16/8	506	21/8	558

The data on the development of the cane given in Table X are also illuminating in this connexion. By about 25 weeks the number of internodes (only fully elongated internodes were considered) was indeed greater by about 1 in Brickfield, which indicates that at least nitrogen did not limit growth in the early stages. The mean internodal length was, however, shorter by 1·7 in., which points to the extension phase of growth being limited by moisture supply. Subsequently the number of internodes differentiated was much less in Brickfield, thus indicating deficiency of nitrogen. The very small increase in leaf growth and the cessation of nitrogen uptake after about 24 weeks has already been referred to. It is significant that after 25 weeks the differences in internodal length consistently diminished, thus indicating that soil moisture was no longer limiting the growth of internodes that developed later. Indeed it seems plausible that the increasing saturation of the heavy soil in Brickfield made soil nitrogen less available to the crop in the later stage.

TABLE X

Number and Length of Internodes

Bhograsan			Brickfield		
Days from sowing.	Mean internode number.	Mean internodal length (in.).	Days from sowing.	Mean internode number.	Mean internodal length (in.).
175	7·4	3·9	170	8·3	2·2
203	11·1	4·1	198	10·2	2·9
231	13·4	4·4	226	11·9	3·4

The much smaller percentage of cane formation in Brickfield can now be understood. By 20 weeks the shoot number in Brickfield was substantially reduced due to deficient moisture supply. Subsequently although the shoot number was equalized, due to higher death-rate in Bhograsan and increase in Brickfield, the latter crop was more heterogeneous, as it consisted of a relatively greater proportion of tillers that developed later. The drought also adversely affected cane formation in the older shoots, as indicated by the smaller rate of increase in plant height and the lower percentage of stem weight in total plant weight.

To sum up: Growth in Brickfield up to the end of July was limited by the comparatively dry weather and the consequent restricted soil-moisture supply which adversely affected leaf-growth, assimilation rate, and development of cane. As a result, although the rate of uptake of nitrogen was diminished, the percentage nitrogen content was higher in Brickfield. Conditions regarding soil-moisture supply then improved and so also did growth. Later, however, nitrogen limited growth, possibly due to excessive accumulation of soil moisture which reduced the supply of available nitrogen. The final yield of millable canes was therefore reduced, due to decrease both in its number and size.

Bhograsan and North Pangarbi

For the first 14 weeks or so tiller production rate was on the whole higher in North Pangarbi. Since dead hearts were removed from North Pangarbi during this period, the mortality rate could not be calculated. Between 14 and 17 weeks tiller production rate was still higher, but the death-rate was also considerably higher, and thus tiller number was more or less equalized in both. Between 17 and 20 weeks higher mortality rate reduced the tiller number in North Pangarbi. Between 20 and 25 weeks although the death-rate was higher in North Pangarbi the production rate was still greater as compared to Bhograsan and the tiller number again equalized. No data were collected from 25 weeks onwards till harvest due to political disturbances during 1942 and lodging in the other treatments as a result of heavy rain and storm in the last week of September.

There was more of leaf growth in North Pangarbi during the first 14 weeks. The leaf-growth rate declined appreciably in North Pangarbi during the next 5 weeks or so. Between 19 and 22 weeks leaf-growth rate was more or less identical in the two fields. Due to political disturbances during August 1942 the sample at the proper time could not be collected. Considering, therefore, the period between 22 and 30 weeks it is seen that the leaf growth rate during this period was considerably slower as compared to Bhograsan, but subsequently it continued at a faster rate.

The percentage nitrogen content was more or less similar in the two cases up to about 22 weeks, and subsequently it appeared to be slightly higher in North Pangarbi. The rate of uptake was higher in North Pangarbi during the first 14 weeks. It has already been seen that tiller as well as leaf growth was higher in North Pangarbi during this period. From 14 to 30 weeks the uptake rate was consistently lower in North Pangarbi, although the percentage nitrogen content did not differ from that in Bhograsan. Distribution of rainfall and other growth data in Table XI indicate a fairly satisfactory correlation between distribution of rainfall and growth, and it seems permissible to conclude that

TABLE XI

Effect of Rainfall in Inches

		Rainfall		
Period.	Month.	Bhograsan.	N. Pangarbi.	Remarks.
First 14 weeks	March	0·66	0·86	Leaf growth and tillering superior in N. Pangarbi.
	April	0·22	0·54	
	May	1·32	0·00	
15th to 19th week	—	4·15	2·10	Leaf growth rate reduced in N. Pangarbi.
20th to 22nd week	—	8·48	9·27	Leaf growth rate almost identical in both.
23rd to 30th week	August	9·28	5·99	Leaf growth rate considerably reduced in N. Pangarbi.
	September*	7·43	13·54	

* 8·6 inches fell about a week before the September sample was taken in N. Pangarbi.

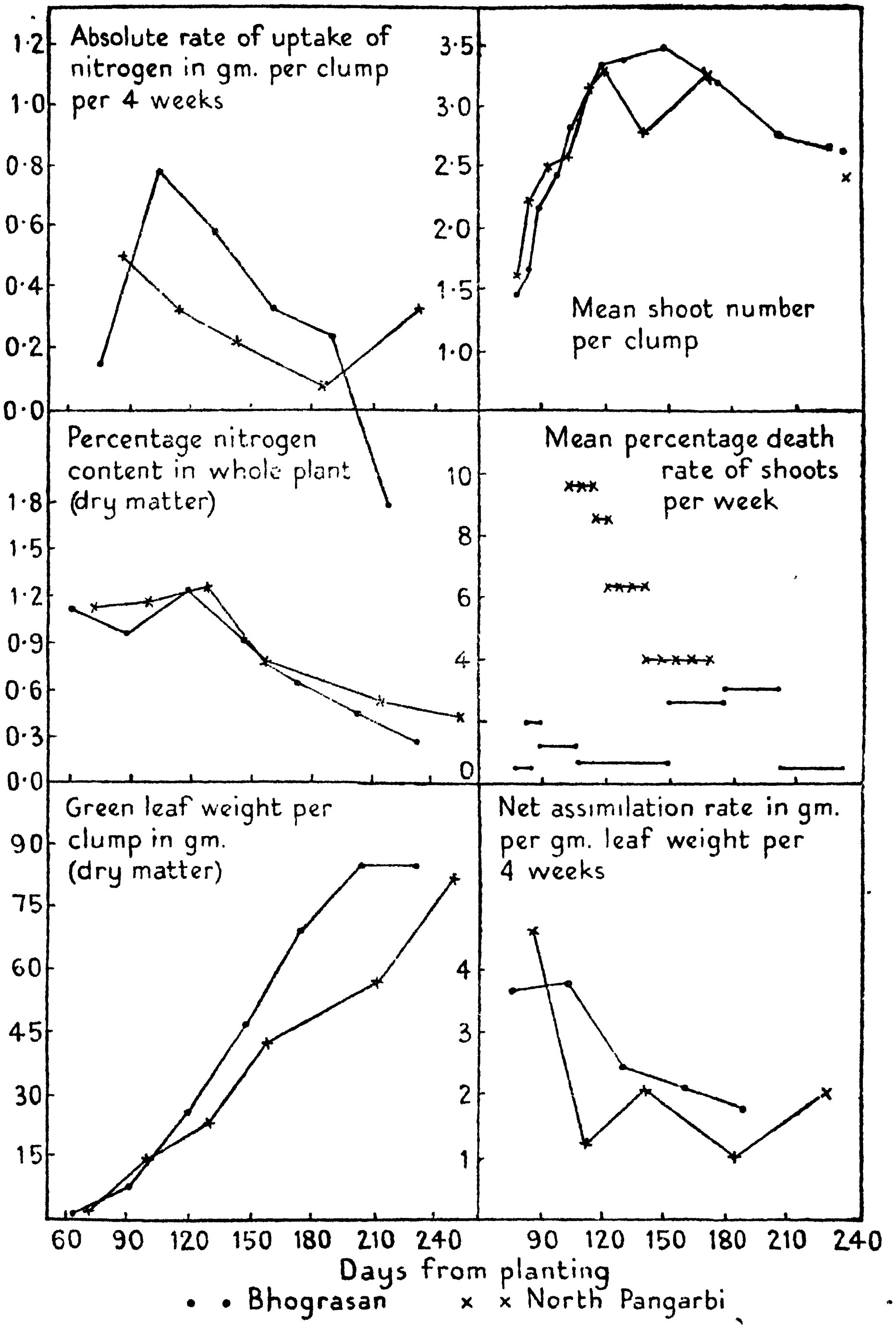

Absolute rate of uptake of nitrogen in gm. per clump per 4 weeks
1·2
1·0
0·8
0·6
0·4
0·2
0·0
Mean shoot number per clump
3·5
3·0
2·5
2·0
1·5
1·0
Percentage nitrogen content in whole plant (dry matter)
1·8
1·5
1·2
0·9
0·6
0·3
0·0
Mean percentage death rate of shoots per week
10
8
6
4
0
Green leaf weight per clump in gm. (dry matter)
90
75
60
45
30
15
Net assimilation rate in gm. per gm. leaf weight per 4 weeks
4
3
2
1
60
90
120
150
180
210
240
90
120
150
180
210
240
Days from planting
• • Bhograsan
× × North Pangarbi

FIG. 4.

the two setbacks in growth suffered by the North Pangarbi crop, viz. during May and August, were due to insufficient water-supply. The rates of elongation of shoots during the monsoon period also indicate the effect of deficient rainfall during August.

TABLE XII

Rates of Shoot-growth

Period.	North Pangarbi.	Period.	Bhograsan.
15/7–31/7	1·29 cm. per day	15/7–12/8	1·72 cm. per day.
31/7–12/8	1·11 ,, ,,	—	—
12/8–1/9	1·44 ,, ,,	12/8–5/9	1·34 cm. per day.
1/9–25/9	1·22 ,, ,,	5/9–4/10	1·43 ,, ,,

The very slow rate of shoot elongation, viz. 1·11 cm. per day during the first half of August in North Pangarbi, is worthy of note. It may also be noted that the difference in final height of the cane in favour of Bhograsan was in a large measure due to the longer internodal length in the latter case.

It is also interesting to note that the net assimilation rate was also lower in North Pangarbi between 15 and 19 weeks and also between 23 and 30 weeks, during which periods water-supply was the limiting factor.

Thus the yield in North Pangarbi suffered, largely due to poor growth of individual cane and, to some extent, to reduction in cane number.

Bhograsan and Chonia

In Chonia the percentage of germination was lower than in Bhograsan. In the 1943 kharif the field was put under maize and consequently the preparation of the field for the following sugar-cane crop was considerably delayed and the usual cultivation schedule was also not carried through. Even at the time of planting the surface soil consisted of clods, and it is felt that germination was comparatively poor on account of the unfavourable physical condition of the soil. Again, 2 weeks after planting there was intermittent rain throughout the succeeding 6 weeks (amounting to 1·40 in.) and this also added to the stiffness of the surface crust. Planting in Bhograsan was done 9 days earlier, and the Bhograsan soil being light, germination was not as adversely affected as in Chonia.

Tillering in Chonia lagged behind Bhograsan from the very beginning and the difference increased with time up to 17 weeks. During the first 13 weeks the production rate was higher and the death-rate lower in Bhograsan. Between 13 and 26 weeks rate of production and death of tillers were more or less identical in the two cases, and thus the difference in tiller number established during the first 13 weeks persisted. Between 26 and 30 weeks the death-rate in Bhograsan was higher and the difference in tiller number amounted almost to nil. Subsequently the death-rate in Chonia was slightly higher. The ultimate difference in the number of mature canes in favour of Bhograsan was, however, more or less proportional to the difference in germination percentage.

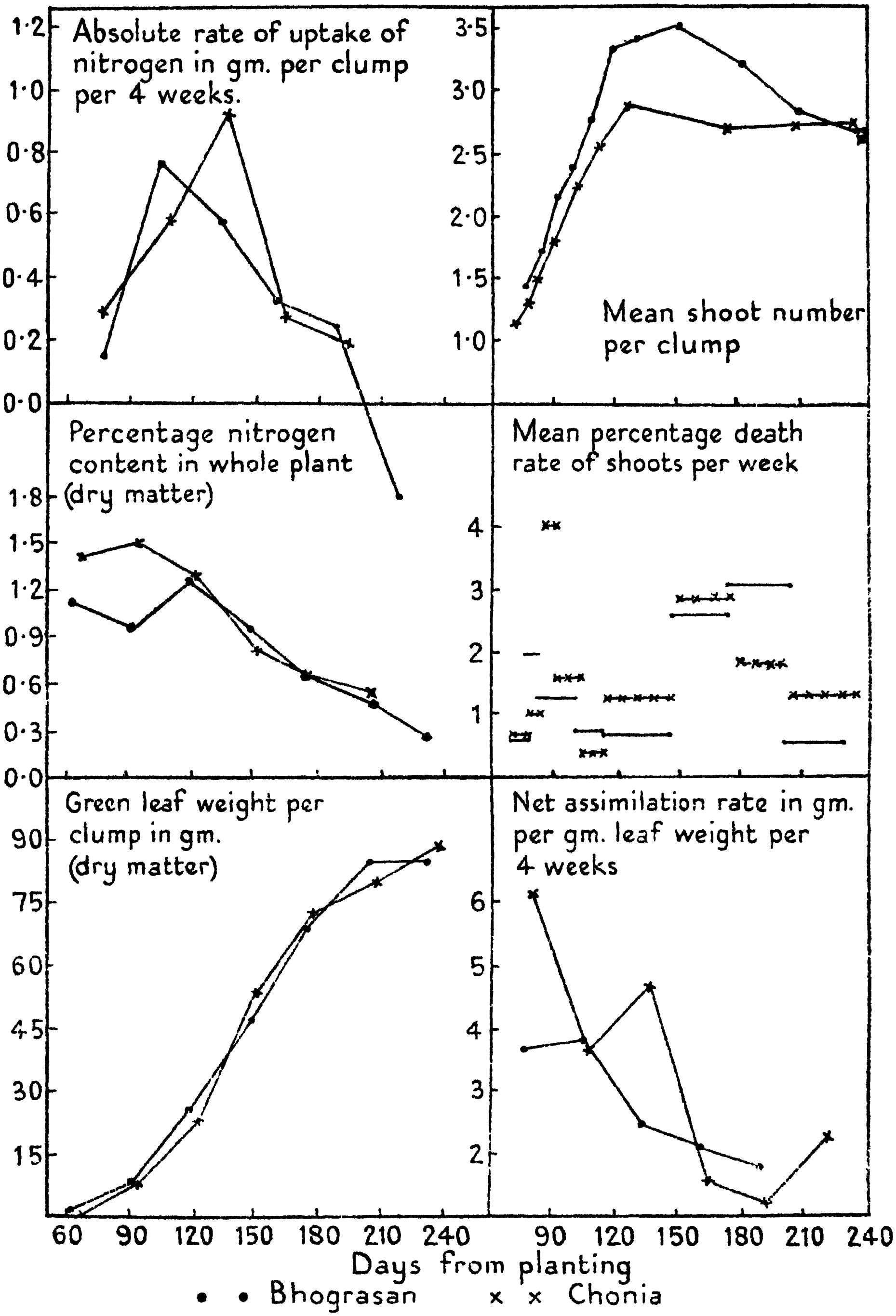

Absolute rate of uptake of nitrogen in gm. per clump per 4 weeks.
1·2
1·0
0·8
0·6
0·4
0·2
0·0
Mean shoot number per clump
3·5
3·0
2·5
2·0
1·5
1·0
Percentage nitrogen content in whole plant (dry matter)
1·8
1·5
1·2
0·9
0·6
0·3
0·0
Mean percentage death rate of shoots per week
4
3
2
1
Green leaf weight per clump in gm. (dry matter)
90
75
60
45
30
15
Net assimilation rate in gm. per gm. leaf weight per 4 weeks
6
5
4
3
2
60
90
120
150
180
210
240
90
120
150
180
210
240
Days from planting
• • Bhograsan
× × Chonia

FIG. 5.

Difference between leaf weights which was established early continued up to about 17 weeks, and equality was attained by about 20 weeks. Between 20 and 25 weeks the leaf weight increased at the same rate in the two cases. Between 25 and 29 weeks leaf growth was less in Chonia but subsequently it was greater, and thus the final leaf weight was more or less identical.

The rate of uptake of nitrogen as well as the percentage nitrogen content indicates that nitrogen was not the factor limiting leaf growth (during the first 17 weeks) and tillering. Very probably it was the stiff nature of the soil surface, referred to earlier, that suppressed emergence of tillers. This would seem to be borne out by the fact that both production and death-rates of tillers were lower during the first 13 weeks, as already noted above. Once, however, the plants were established their growth continued at the same rate as, or even higher than, that in Bhograsan.

Unfortunately the last sample in Chonia was accidentally destroyed by fire and its nitrogen content could not be estimated. From the general trend of the curve of nitrogen uptake and the percentage nitrogen content it appears, however, that nitrogen did not become unavailable at later stages as it happened with heavy soils in Harpur Jhilli and Brickfield. It may be noted that Chonia is not situated in a low-level area and therefore there is adequate drainage. Actually the average size and weight of an individual cane was larger in Chonia, but the total yield suffered because of the smaller number of plants per unit area as a result of poor germination and setback to tillering in the early stages.

DISCUSSION

The outstanding effects of environmental conditions on growth of sugar-cane in the four seasons under consideration may be summarized as follows:

Field.	Developmental characters affected (as compared with Bhograsan).	Factors responsible.
Nepali . .	Tillering and final cane number reduced.	Deficiency of nitrogen in the early stages.
Harpur Jhilli .	Tillering, leaf growth, and final cane number reduced.	Deficiency of nitrogen after 13 weeks due to excessive soil moisture.
Brickfield .	Tillering, leaf and stem growth, net assimilation rate, and cane number and size reduced.	Drought in the early stages; deficiency of nitrogen later due to excessive soil moisture.
Chonia . .	Germination, tillering, and final cane number reduced.	Stiff surface crust due to insufficient preparation of land.
North Pangarbi	Reduction in leaf and stem growth, net assimilation rate, cane number, and size.	Partial drought in May and August.

Reduction in yield as compared to that of Bhograsan appears, therefore, to be due to (i) poor germination due to insufficient preparation of land, (ii) partial drought, (iii) deficiency of nitrogen, and (iv) unknown soil factor.

Factor (i) need not be considered.

As compared to Bhograsan, tiller production in both Nepali and Harpur Jhilli suffered in the early stages as a result of nitrogen deficiency, whereas in Brickfield and North Pangarbi tiller number was reduced later, due to partial drought. It would seem that both nitrogen and moisture influence tillering, but the latter apparently controls growth and survival of the tiller and not its initiation. Since difference in tiller number established as early as 17 weeks after planting reflect upon cane number at harvest, it is obvious that tillers developed during this period contribute substantially to yield; the possibility that the effects of borers might alter this relationship later is, however, not ruled out. The maximum tiller number is usually attained by the end of the hot weather (early July), but it does not appear likely that the usual high temperature and low relative humidity during this period have a markedly adverse effect upon tillering, at least in the case of Co 313. More likely, nitrogen has a relatively preponderant effect upon tillering. The possibility of tillering benefiting by irrigation (if possible) in the case of the usual hot and dry weather during this phase is not excluded, but the effect of increased soil moisture on the availability of nitrogen may also be involved.

With the onset of the monsoon and the accompanying increase in relative humidity stem internodes begin to elongate. At this stage demand for water increases considerably because water is required for the extension phase of growth as well as for the necessary turgidity of leaves for active assimilation. Deficient rainfall is, however, likely to affect adversely assimilation rate and also leaf and cane growth. The variety Co 313 appeared to be susceptible to drought at this stage, as it suffered in all these respects in North Pangarbi and Brickfield where drought occurred.

With uniform distribution of rainfall and in absence of waterlogging it would then seem that Pusa soils can produce per acre at least 20 tons of millable cane under the usual rotations and even without addition of nitrogen at the time of planting.

The importance of uniform distribution of rainfall raises the difficult issue of obtaining varieties more tolerant to partial drought than Co 313. It was seen how the net assimilation rate was depressed by the partial drought in Brickfield and North Pangarbi. It is therefore tentatively suggested that assimilation rate would serve as a sensitive and suitable index for testing tolerance of varieties to drought. In fact, the writer (1943 and 1948) has observed that in a few sugar-cane varieties that he had occasion to study in a preliminary way (though not from the view-point of drought tolerance) the assimilation rates (as estimated by Sach's half-leaf method and by difference between the total carbohydrate contents of morning and afternoon samples of leaves) differed appreciably. This observation accords with Watson's view (1947) that the net assimilation rate varies between varieties of the same species. It conflicts with Heath and Gregory's generalization (1938) about the constancy of the net assimilation rate. Watson (1947), however, accounts for the constancy of net assimilation rate, observed by Heath and Gregory, by postulating that species of higher net assimilation rates may have been associated with less favourable environments.

Crowther (1934), in cotton, and Watson (1947), in wheat, sugar-beet, and potato, found that yield was more closely related to leaf area than to net assimilation rate. According to Crowther (1934 and 1937) nitrogen and water-supply had no effect on the net assimilation rate in cotton in the Sudan, whereas in Egypt both nitrogen and spacing affected it. Very likely the rate of carbon assimilation was not depressed on account of the leaf turgidity being little affected by the different levels of irrigation used. Crowther interpreted the effect in Egypt as due to differential shading brought about by nitrogen and spacing. Tiver and Williams (1943) found, on the other hand, that growth of the flax plant was depressed by low-moisture treatment due to a depression of the net assimilation rate. Petrie and Arthur (1943) also found a similar effect of drought in the case of the tobacco plant. To sum up, although observations, so far reported in the literature, on the influence of environmental factors on the net assimilation rate and on its relation to yield do not lead to a consistent view, it appears to the writer that a systematic study of the relation between assimilation rate of sugar-cane varieties and environmental conditions (particularly restricted moisture supply) might repay the effort.

It is conceivable that in spite of partial drought during July the Brickfield crop would have subsequently made up in growth had it not suffered from nitrogen deficiency due to excessive accumulation of soil moisture. In view of the existence of extensive low-lying areas in North Bihar it is worth considering whether varieties more tolerant (than Co 313) to this soil condition can be obtained. Since leaf growth was conspicuously checked under these conditions it appears necessary to ascertain, in the first instance, whether some adverse soil condition, for example, reduced aeration, checks the supply of available nitrogen or inhibits root activity. A comparative study of the rate of leaf production in different varieties under this condition may also prove useful in this connexion. It is worth considering, besides, whether a variety with a relatively smaller requirement of nitrogen, if there be any, might not be more suitable to these conditions.

In the case of Nepali nitrogen limited growth only during the first 25 weeks, and subsequently it was in sufficient supply. The analysis of weather data does not throw any light on this peculiar soil condition. It may be noted that nitrogen applied as castor cake (in nitrogen treatment in Nepali) increased tillering during the first 12 weeks, but subsequently its effect disappeared completely. From the data at hand it is not possible to suggest the nature of the factor that adversely affected soil nitrogen. A close study of soil conditions is indicated, for it is in such cases that ameliorative measures may be expected to raise yields by ensuring efficient utilization of nitrogen.

SUMMARY

In North Bihar in north-east India is located the second largest white-sugar belt in India, which is unique in the respect that sugar cane is grown there as

an annual crop entirely under rain-fed conditions. The average yield of millable cane approximates to about 15 tons per acre, and response to manuring with nitrogen and phosphorus is inconsistent.

Results presented in the paper relate to developmental observations on the variety Co 313 carried out during four seasons in six different fields (unmanured control plots) at Pusa, where conditions are fairly representative of the tract. Data on growth in the field (Bhograsan) which gave the highest yield are taken as a base-line for comparison with growth in other fields. The relation of growth to variation in final yield is discussed.

It was observed that the tillering phase which is nearly completed (during the hot weather) by the time the monsoon sets in, and the intensity of which influences in a large measure the yield of mature cane, was not so much influenced by the intensity of the hot weather as by the nitrogen supply.

During protracted periods of scanty rainfall during the monsoon growth suffered as a result of depression in the rates of assimilation and leaf growth. It is suggested that for selecting varieties more tolerant than Co 313 to partial drought the assimilation rate might serve as a sensitive and suitable index.

Waterlogging in low-lying areas also checked growth by depressing the rate of uptake of nitrogen and leaf growth. It is suggested that further investigation on the influence of waterlogging on the availability of nitrogen, and on root activity and rate of leaf production in different varieties, might point to a suitable index for selecting varieties adapted to this condition.

Growth analysis also pointed to another soil condition, not identified as yet, under which availability of nitrogen was temporarily reduced. It is believed that its identification might indicate the means of ensuring more efficient utilization of nitrogen.

ACKNOWLEDGEMENT

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Physiological and Ecological Studies in the Analysis of Plant Environment

V. An Assessment of the Factors controlling the Distribution of the Bluebell (*Scilla non-scripta*) in Different Communities

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With five Figures in the Text

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INTRODUCTION

THE four previous papers in this series (Blackman and Rutter, 1946, 1947, 1948, 1949) have been concerned essentially with an analysis of the relative importance of the light factor and the levels of mineral nutrient supply in determining the distribution and growth of the bluebell (*Scilla non-scripta*) in woodland. It has been demonstrated for a number of woodland sites that where the canopy is closed the variations in bluebell density are largely determined by the overall seasonal differences in the degree of shading. Subsequently it was shown by field and pot experiments, where the light intensity was varied by means of screens, that the bluebell does not demand a high status of mineral nutrition and can in no way be regarded as an 'obligate shade' plant in which the assimilation rate and growth rate reach maxima at low levels of light intensity. In the first place, over the range of 0·1 to full daylight, the net assimilation rate is proportional to the logarithm of the light intensity. Secondly, though the leaf-area ratio increases in logarithmic

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proportion to the degree of shading, the growth rate does not approach the maximum until the light intensity exceeds 0·6 daylight. It is therefore clear that in woodland the light intensity is likely to be the major factor controlling growth. In fact, so far as conditions of shade are concerned, the bluebell reacts like a typical 'sun' plant.

In the present paper an attempt has been made to assess the importance of factors other than light intensity which may play a part in controlling the distribution of *Scilla non-scripta*, both within and without woodland. In such an assessment many problems are involved and a certain diffuseness cannot be avoided. It is inevitable that the lines of investigations which have been undertaken are diverse and have arisen either out of the early light experiments or from a general consideration of the autecology of *S. non-scripta*.

Although the bluebell is a very common woodland plant, yet in some areas in the south-west of England, in Wales, and on the western coast of Scotland, *S. non-scripta* is often a component of grassland communities, and is also found in association with *Pteridium aquilinum*. This occurrence in such open situations has hitherto been explained on the basis that the sites had been woodland in the past, and that the bluebells have survived the felling of the trees. On the other hand, apart from the evidence of the present investigations, other observations cast doubt on this explanation. In islands off both the Brittany and Welsh coasts, bluebells can be found growing on rock ledges and in grassland and bracken communities where the shallowness of the soil and the full exposure to the wind could not have allowed tree growth in the past. Similarly, *S. non-scripta* is also found at altitudes well above the existing tree line, e.g. at 1,800–2,000 ft. in Wales (Cader Idris), the Lake District, and in Scotland (Ardgarten Forest).[1]

Against this background it seemed evident that the factors controlling the growth of *S. non-scripta* in grassland ought to be investigated. In considering the design of the experiments it appeared significant that at least in many of the localities examined the areas where the bluebells grew in the open were either not grazed or only very lightly grazed. This conjunction suggested that biotic factors of trampling or grazing might limit the distribution in such communities.

In the previous investigations no consideration was given to the possibility that in different communities ecotypes of *S. non-scripta* might have arisen, and that these types would exhibit different responses to the major factors controlling growth. Accordingly, comparative experiments have been made with bulbs collected from a number of contrasting habitats, in order that any differential effects of varying light intensity on growth and development could be studied and any potential difference in the ability to compete with grasses assessed.

A further problem which has been given detailed consideration relates to the possibility that when plants are grown under varying conditions of light intensity or with different levels in the nutrient status of the soil, then the effects of these variables may carry over into the *subsequent* year and cause variations in seasonal growth. The analysis of this problem has in turn necessitated a study of the relationship between seasonal growth rate and varying bulb size.

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[1] Private communications from Dr. J. H. Salter, Dr. Muir, and Prof. T. G. Tutin.

The present paper, therefore, consists in the main of four aspects relating to the analysis of the autecology of *S. non-scripta*. In the case of the experiments concerned with the nature of the competition with grasses and the comparison of ecotypes, the results cannot be differentiated since bulbs from different communities have been used in the grassland experiments.

EXPERIMENTAL RESULTS

I. *The Nature of the Competition in Grassland*

In considering the nature of the competition between *S. non-scripta* and the constituent species of grassland communities it seemed probable that the major factors involved would vary with the dominant species of grasses in the sward. In consequence, during the two years in which experiments were carried out on an alluvial gravel soil at the Field Station, Slough, Bucks., the bulbs were planted in almost pure stands of different grass species which had been sown 3 or 4 years earlier. In 1939 the three swards were dominated by *Phleum pratense*, *Festuca pratensis*, and *F. rubra* respectively. In each sward dormant bulbs of widely varying sizes were planted in the autumn. The large bulbs had already reached a size when inflorescences are normally produced, while the small bulbs were estimated to be not more than 2 or 3 years old.

The method of planting the mature bulbs was to cut with a metal tube a cylindrical core from the turf, 2 in. in diameter and 4 in. deep. The bulb was placed in the bottom of the hole, and the core of turf replaced and pressed down. For the small bulbs a core of turf 1 in. in diameter and 2 in. deep was cut out; it was broken about ½ in. below the top and the bulb was put between the two pieces. These pieces were then placed together again and the whole was slid into a piece of glass tube, which was open at each end, also 1 in. in diameter and 1–2 in. long. The tube containing the turf and the bulb was dropped into the hole in the plot; the turf was pressed well down, but the glass allowed to project 0·5–1·0 in. above the level of the ground. This procedure was adopted so that during the course of the experiment the small bulbs could be located, for without some method of marking it would have been almost impossible to find the bulbs at the end of the growing season when the leaves had died back. Moreover, the tubes facilitated the cutting of the grass without defoliating the bluebell plants. On general considerations it seemed unlikely that the presence of the tubes would introduce serious errors.

The success with which bluebells can compete with grasses is likely to be related to the rate of growth and the ultimate height of the different species. Accordingly, attempts were made to alter the rate of growth and height in the following ways. Half the plots were treated with ammonium nitrate applied in solution at a rate equivalent to 50 lb. of nitrogen per acre, while the others were left untreated. In addition half the plots were kept short by cutting while the other half were left to grow freely. When the grass was cut, care was taken not to cut or damage the bluebell leaves. Differential grazing like this differential cutting is not likely to occur in nature, but might if the bluebells were readily distinguished in the sward, and were unpalatable to the grazing animal. These two types of treatment were combined factorially, giving four treatments in all, viz. not cut plus nitrogen, not cut, cut plus nitrogen, cut. For each grass the treatments were in threefold replication. Unfortunately the mature bulbs were so badly attacked by *Penicillium cyclopeum* that they were discarded. But reliable data were obtained for the small bulbs, of which 10 were grown on each plot.

Without additional nitrogen there was no significant difference between cut and uncut plots (Table I), but in the plots which had received nitrogen, cutting the grass caused a significant increase in the growth of the bluebells. On the uncut plots the application of nitrogen significantly decreased the growth, but on the cut plots a significant increase in growth was brought about by nitrogen.

This interaction is highly significant, and it is put forward that in the uncut plots any positive effect of nitrogen in increasing the growth of the bluebell is outweighed by an indirect negative effect of nitrogen in increasing the growth and height of the grasses, and thereby adversely affecting the bluebell because of the increased shading. In consequence, it is only when there is little or no shading of *S. non-scripta* on the defoliated plots that nitrogen causes a gain in weight.

TABLE I

Experiment XI, 1939. The Interaction of Selective Cutting and Nitrogen Supply on the Seasonal Growth of Seedling Bulbs of Scilla non-scripta *in Grassland*

Dry weight of 10 bulbs at end of season (grammes)	Nitrogen.	No nitrogen.	Mean.
Sward cut	0·776	0·640	0·708
Sward not cut	0·575	0·663	0·619
Significant difference ($P = 0·05$) .	0·071		0·051

Since the effect of nitrogen is positive on the cut plots and negative on the uncut plots, the mean effects of nitrogen are small, but there are differences for the grass species. With *Phleum pratense*, while there is a marked increase due to nitrogen on the cut plots, there is little or no decrease on the uncut plots. In consequence the mean effect of nitrogen on *S. non-scripta* is significant in this species (Table II). The overall effect of grass species is not, however, significant.

The initial dry weight of the ten bulbs planted in the previous autumn was 0·279 g., and it is clear from Tables I and II that these very small plants have

TABLE II

Experiment XI, 1939. The Interaction of Selective Cutting, Nitrogen Supply, and the Dominant Species of Grass on the Seasonal Growth of Seedling Bulbs of Scilla non-scripta *in Grassland*

Dry weight of 10 bulbs at end of season (grammes)

Dominant species.	Sward cut.		Sward not cut.		Means of cutting treatments.	
	Nitrogen.	No nitrogen.	Nitrogen.	No nitrogen.	Nitrogen.	No nitrogen.
Phleum pratense	0·821	0·578	0·648	0·674	0·734	0·626
Festuca pratensis	0·774	0·747	0·522	0·646	0·648	0·697
Festuca rubra	0·735	0·594	0·554	0·669	0·643	0·631
Significant difference (*P* — 0·05)					0·088	

been able to double their weight, even under the greatest degree of competition which this experiment produced; that is to say, on plots which received nitrogen and were not cut.

Further experiments on competition with grasses were carried out in 1940 on swards consisting either of *Dactylis glomerata* or *F. rubra* sown 4 years previously. In each experiment there were three treatments. Bulbs were either planted in the sward or in the soil after the surface turf had been stripped, while subsequently half the sward plots received ammonium nitrate at a rate equivalent to 50 lb. of nitrogen per acre. It was therefore possible to compare the growth of plants with and without competition from either of the grasses.

In these experiments a comparison was also made of bulbs collected from three different sites, so that the possible differences in these populations might be investigated. It is proposed to refer to these populations as 'strains', since it will be shown subsequently that they differed significantly in a number of physiological characteristics. Strain I came from an open oak (*Quercus robur*) wood at Warfield, Berks., when there was a thin understorey of coppiced hazel (*Corylus avellana*); strain II was collected from an ash wood with a closed canopy at Gravetye, Kent, and strain III from a plantation of larch (*Larix japonica*) at Shurlock Row, Berks. Strains I and II were represented by two sizes of bulb, while those of strain III were very much smaller. Taking first the large bulbs, the initial mean weights for strains I and II were 1·38 and 1·28 g. per bulb, while the corresponding figures for the medium-sized bulbs were similar, viz. 0·37 g. The small bulbs of strain III averaged 0·037 g. On account of the discrepancies between the initial weights of the different classes of bulbs, the ratio of final to initial weight has been used as the measure of seasonal growth. On this basis it is seen from Table III that the differences between the three treatments are highly significant when the data are transferred to a logarithmic scale. Growth was least on the plots receiving nitrogen where the plants only maintained their initial weight, while the increase was greatest on the stripped plots, i.e. without competition from *D. glomerata.*

TABLE III

Experiment XII, 1940. Seasonal Growth of Scilla non-scripta *in a* Dactylis glomerata *Sward*

Treatment.	Ratio of final to initial weight. Ratio.	Ratio of final to initial weight. Log. transformation.
Surface turf removed	1·664	1·180
Untreated sward	1·386	1·118
Nitrogen added to sward	1·116	1·025
Significant difference (log. scale $P = 0·05$)		0·082

There were also significant differences in the growth made by the different kinds of bulb (Table IV). On the grass plots the large-sized bulbs of both strains I and II failed to maintain, or only just maintained, their initial weight, and on the bared plots the large strain II bulbs gained little during the season. On the other hand, the medium-sized bulbs made more growth, probably because they did not set seed. The small bulbs made only a slight gain over their initial weight. In only one case did the plants double their initial weight, namely the medium-sized bulbs of strain I on the bared plots.

TABLE IV

Experiment XII, 1940. The Effect of Strain and Bulb Size on the Seasonal Growth of Scilla non-scripta *in a* Dactylis glomerata *Sward*

	Ratio of final to initial weight.				
Source and bulb size.	Turf removed.	Untreated sward.	Nitrogen added to sward.	All treatments.	All treatments (log. scale).
Strain I					
Large	1·57	1·09	0·96	1·21	1·061
Medium	2·74	1·76	1·62	2·04	1·283
Strain II					
Large	1·13	1·00	0·78	0·97	0·965
Medium	1·82	1·80	1·13	1·58	1·182
Strain III					
Small	1·06	1·28	1·09	1·14	1·047
Significant difference (log. scale $P = 0·05$)					0·075

Experiment XIII was similar in design to expt. XII, save that the dominant grass was *F. rubra* in which medium-sized bulbs of strains I and II and small bulbs of strain III had been planted. On account of the limited area available, the replication was only threefold, and none of the effects are significant. The results, however, set out in Table V are similar to those of expt. XII. The growth of flowering bulbs is apparently better on bared soil than in the sward; on the former the plants just doubled their weight, but on the latter failed to do so. Under each set of conditions the small bulbs lost weight during the season.

TABLE V

Experiment XIII, 1940. Seasonal Growth of Scilla non-scripta *in a* Festuca rubra *Sward*

Strain of *S. non-scripta*.	Ratio of final to initial weight. Turf removed.	Untreated turf.	Nitrogen added to turf.
Strain I, medium-sized bulbs	2·28	1·49	1·67
Strain II, medium-sized bulbs	1·72	1·38	1·41
Strain III, small-sized bulbs	0·89	0·85	0·57

II. *The Effects of Defoliation and Trampling on the Seasonal Growth of the Bluebell*

In designing experiments to investigate the effects of grazing animals on the growth of the bluebell, there are clearly two main considerations. Firstly, animals may defoliate the plants and, secondly, if they are unpalatable, they may only trample on them. To simulate the effects of grazing, the leaves, and the inflorescence if present, were cut off with a pair of scissors. To imitate the effects of trampling it seemed that an approximation to the mechanical action of a hoof could best be achieved by walking backwards along the row of plants, placing the heel on top of each plant. In both sets of experiments the dormant bulbs were planted in the autumn on a gravel soil in rows some 12 in. apart, with 6 in. between each bulb.

The effects of defoliation on seasonal growth. Experiments were carried out at Slough in both 1939 and 1940. Experiment XIV was designed to assess the influence of the frequency and time of defoliation on the changes of weight during the period of seasonal growth. Some thirty mature bulbs were planted on each plot in the autumn of 1938, and in the following spring the leaves and the inflorescence, if present, were cut off (1) before the inflorescence had emerged above ground, (2) before the flower buds opened, and (3) when the plants were in full flower. In addition, there were two sets of double defoliation; the leaves were cut at stages 1 and 2 or 1 and 3.

TABLE VI

Experiment XIV, 1939. The Effects of Defoliation at Different Stages of Development on the Seasonal Growth of Scilla non-scripta

Stage of development at time of defoliation.	Final dry weight per bulb (g.).
Single defoliation	
(1) Before inflorescence emerges	2·09
(2) Before flower buds open	1·67
(3) At flowering	2·01
Double defoliation	
(1) Before inflorescence emerges and (2) before flowering	1·08
(2) Before inflorescence emerges and (3) at flowering	1·26
Control	3·62
Significant difference ($P = 0·05$)	0·44

From the results of Table VI it is clear that all cutting treatments have markedly depressed growth. When the leaves are cut off, the bases continue to grow a little, but no new leaves are produced, and there is in consequence the greatest decrease in assimilatory tissue with the later cuttings. If the first cutting is followed by a second later in the season, the effect is even more pronounced.

The mean initial dry weight was 4·01 g. and on all the plots the bulbs were attacked by *P. cyclopeum*. In consequence, even the control plants showed a slight decrease in weight over the season, whereas on the evidence of other experiments they might have been expected to double their weight. The results, however, suggest that under more favourable circumstances the once-cut plants would have failed to increase much in weight, and that a double defoliation would certainly have caused a loss in weight.

In 1940 another similar experiment was carried out, but, in this case, single bulbs in pots were employed. The initial dry weight was calculated for each bulb separately, and the analysis of variance is based on the ratio of final to initial dry weight. The results are shown in Table VII.

TABLE VII

Experiment XV, 1940. The Effects of Defoliation at Different Stages of Development on the Seasonal Growth of Scilla non-scripta

Stage of development at time of defoliation.	Ratio of final to initial dry weight.
Single defoliation	
(1) Before inflorescence emerges	0·71
(2) Before flower buds open	0·89
(3) At flowering	1·10
Double defoliation	
(1) Before inflorescence emerges and (2) before flowering	0·77
(2) Before inflorescence emerges and (3) at flowering	0·69
Control	1·24
Significant difference (*P* = 0·05)	0·30

In addition to the severe effect of two cuttings, this experiment shows that the effect of a single cutting is greater the earlier it is made. The pots tended to dry out very rapidly and the effects of frequent wilting must be taken into account. This water shortage is reflected in the small increase in weight on the controls, but here again there was some attack by *P. cyclopeum*. A similar experiment was made in 1940 with bulbs planted in open ground, and the greater effect of an earlier defoliation is again shown in Table VIII, although the difference between the two times of cutting is not quite significant.

The effects of trampling. Three experiments on the relationship between the incidence and intensity of trampling were carried out during 1939 and 1940. In expt. XVII, as soon as the leaves were well above ground the appropriate plants were 'trampled' at 7-, 14-, 21-, and 28-day intervals. Unfortunately the incidence of *P. cyclopeum* was widespread and even on the control plots

TABLE VIII

Experiment XVI, 1939. The Effects of Defoliation on the Growth of Scilla non-scripta

	Final dry weight per bulb (g.).
Control	1·231
Leaves cut at ground level at emergence of inflorescence	0·636
,, ,, ,, ,, late flowering stage	0·851
Significant difference (*P* = 0·05)	0·267

little growth was made during the season. Nevertheless, from the data given in Table IX it is clear that frequent trampling has had a very adverse effect.

TABLE IX

Experiment XVII, 1939. The Effects of the Frequency of Trampling on the Seasonal Growth of Scilla non-scripta

Treatment.	Final dry weight per bulb (g.).
Control	3·04
Trampled every 7 days	1·24
,, ,, 14 ,,	2·19
,, ,, 21 ,,	2·08
,, ,, 28 ,,	2·32
Significant difference (*P* = 0·05)	0·43

In 1940 a similar experiment was undertaken in which there were two intensities of trampling, viz. trampling once in 10 days and trampling once in 20 days. Superimposed on these treatments were two stages at which trampling was initiated; in the first the trampling was begun soon after the appearance of the leaves, i.e. on May 8, and in the second it was started 20 days later. In addition, the plots were split so that bulbs of strains I and II could be included in the experiment.

TABLE X

Experiment XVIII, 1940. The Effects of Trampling on the Growth of Scilla non-scripta

	Final dry weight per bulb (g.). Intensity of trampling.			Period means.	Period means (log. scale).
Period of trampling.	Control.	10 days.	20 days.		
Trampling started May 8	—	0·583	0·695	0·726	0·86
,, ,, ,, 28	—	0·925	0·957	0·980	0·99
Intensity means	0·981	0·753	0·826	—	—
Intensity means, log. scale	1·00	0·86	0·91	—	—

Significant difference (*P* = 0·05) between period means on log. scale = 0·05
Significant difference (*P* = 0·05) between intensity means on log. scale = 0·06

Table X shows that both the intensity of trampling and the stage at which trampling is initiated produce significant effects on seasonal growth. The deferment of trampling by 3 weeks has produced a smaller adverse effect. Since the mean initial weight in the previous autumn was 0·452 g., even the most intensive trampling has not prevented the plants from gaining weight during the season.

Although at first the bulb weights of strains I and II were similar (0·459 and 0·444 g.), by the end of the season there was evidence of an interaction between strain and the period of trampling. Extending the period has had a greater effect in the case of strain I (see Table XI). As strain I produces leaves earlier in the season than strain II (see p. 500), early trampling is likely to cause more damage to the plants with the most advanced leaf formation.

TABLE XI

Experiment XVIII, 1940. The Effects of the Period of Trampling on the Seasonal Growth of Two Strains of Scilla non-scripta

Trampling treatment.	Final dry weight per bulb (g.). Untransformed data.		Transformed data (log. scale).		Sig. diff. (*P* = 0·05).
	Strain I.	Strain II.	Strain I.	Strain II.	
Trampling started May 8	0·83	0·63	0·91	0·78	0·07
" " " 28	1·19	0·77	1·09	0·89	
Mean	1·01	0·70	1·00	0·84	0·05

III. *The Differential Reaction to Varying Light Intensity of Bluebell Plants from Different Habitats*

The experimental methods adopted for the analysis of the effects of shading on the seasonal growth and development of the different strains of the bluebells were similar to those described in detail in the previous papers (Blackman and Rutter, 1947, 1948). Graded bulbs of uniform size were planted on a cultivated gravel soil in the autumn, and as soon as the leaves emerged above ground in the following spring the appropriate plots were shaded by means of light wooden frames covered either with butter-muslin or sheets of perforated zinc. During the growing season random samples were taken from each plot; the plants were divided up into bulbs plus roots, leaves, and inflorescence; the separate parts dried in an oven and weighed. Before the leaves were dried a sub-sample was taken for the determination of the ratio of leaf area to leaf weight and by means of this ratio the leaf area per plant was estimated.

From these data it is possible to calculate the effects of varying light intensity on the changes in plant weight, leaf-area ratio, and net assimilation rate of each strain.

In the largest of the experiments, viz. expt. V, 1940, besides three levels of light intensity (daylight and 0·68 and 0·22 daylight) there were eight nutrient treatments consisting of nitrogen, phosphorus, and potassium, alone and in combination, and three strains of bluebell. Only the interactions between the strains and varying light intensity will be considered in this paper, since the mean effects of light intensity and nutrient supply have already been discussed (Blackman and Rutter, 1947, 1948) and all the strains have given a similar response to the nutrient treatments.

Two of the strains, I and II, have already been described on page 491, while the third strain (strain IV) consisted of bulbs purchased from a bulb farm in Gloucestershire, where the plants had been raised in the open. Because of the large number of bulbs required, it was not possible to ensure that the mean bulb weight at the time of planting in the autumn was exactly the same for each strain. The mean bulb weights of strains I and IV were similar—0·92 g.—but for strain II the weight was 0·79 g. To eliminate these initial differences two other methods of comparison were adopted. Firstly, over the period April 23 to May 28, that is shortly after the leaves had appeared to when the leaf area was at a maximum, the efficiency indices were compared. Secondly, the weights on July 17 relative to the weights at planting were examined. The results in Table XII and Fig. 1 show that there were considerable differences between the rates of growth of the strains; strain I had the highest and strain II the lowest rate.

TABLE XII

Experiment V, 1940. The Effects of Varying Light Intensity on the Growth Rate of Different Strains

Percentage increase in dry weight per day, April 23–May 28.

Light intensity.	Strain I.	Strain IV.	Strain II.
1·00 daylight . . .	3·32	3·01	3·03
0·68 ,, . . .	3·32	3·05	2·95
0·22 ,, . . .	2·30	1·64	1·61
Mean	2·98	2·57	2·53
Significant difference (*P* = 0·05) = 0·19			

An analysis of variance of the data of Fig. 1 after conversion to a logarithmic scale reveals that the reactions to changes in light intensity do not differ significantly between strains.

A further experiment (expt. XIX) in which five strains were grown at four levels of light intensity was undertaken in 1940. In addition to the four strains already described, there was a fifth collected from a clearing in a beech wood at Yattendon, Berks., where there was a dense stand of coppiced hazel. The four light treatments were replicated five times, while 10 bulbs of each of the five strains constituted sub-plots. Each lot of 10 bulbs was weighed before planting in 1939, and the dry weights were calculated after the percentage dry matter of a sub-sample had been determined. The mean initial weights of 10 bulbs of the five strains were: strain I, 5·66±0·26 g.; strain II, 5·96±0·11 g.; strain III, 6·60±0·28 g.; strain IV, 6·22±0·09 g.; and strain V, 7·01±0·11 g.

All the bulbs were dug up on July 17, and an analysis of variance has been carried out on the ratio of the final to initial weight after transformation to a logarithmic scale. This analysis shows that a reduction from daylight to 0·68 of daylight caused no significant fall in the ratio over all five strains (2·76 as against 2·78), but that at the two lower light levels of 0·22 and 0·11 daylight, the mean ratios have been significantly depressed, viz. 2·10 and 1·53 respectively. As in the previous experiment, there were marked differences between the strains. Over all light intensities, strain V made the maximum growth (mean ratio 3·20), followed by strain I (2·71), strain III (2·24), strain II (1·74), and strain IV (1·69).

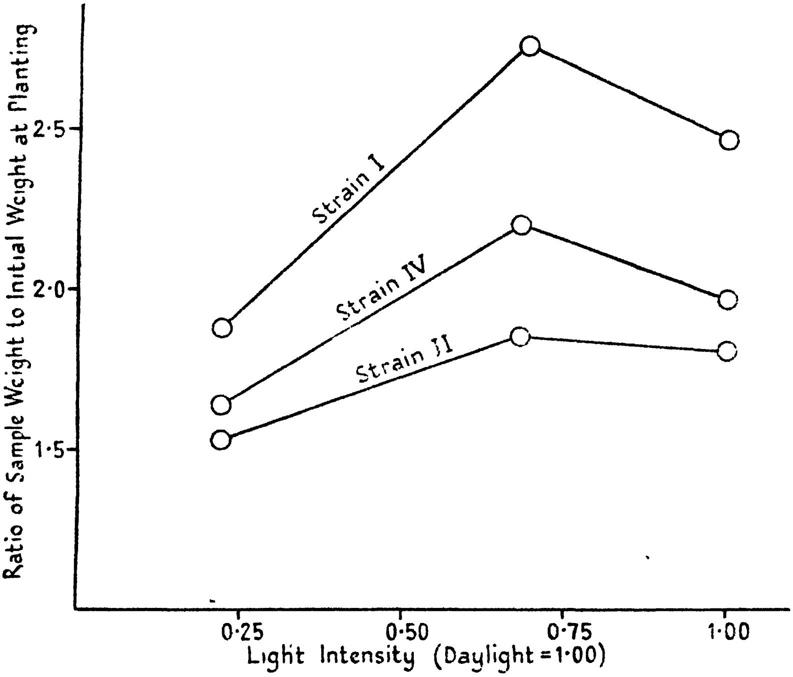

FIG. 1. Experiment V. The effects of varying light intensity on the seasonal growth of different strains of *Scilla non-scripta*.

This difference between the strains is brought out clearly in Fig. 2, where the changes in the ratio for each strain have been plotted against the logarithm of the light intensity. For strains I and III–V, curves of the form $y = a+bx+cx^2$ have been fitted to the data, but for strain II a better fit was obtained with a linear regression.

From these equations the extrapolated light values at which no growth would have been made during the season can be calculated. They are 0·087, 0·074, 0·071, 0·066, and 0·05 of daylight for strains IV, III, V, I, and II respectively.

A further analysis of the differences in the growth rate of strains can be obtained from the data of expt. V. It will be recalled that for the first sampling period besides estimates of the relative growth rates of the three strains under the different light intensities the leaf areas were also measured. It is thus possible to calculate the corresponding net assimilation rates between the two sampling occasions and also the mean ratio of leaf area to plant weight—that is the leaf-area ratio of Briggs, Kidd, and West (1920). The assimilation rates have been calculated according to the usual formula (Gregory, 1926; Williams, 1946), while the leaf-area ratio is the arithmetic mean for the two sampling occasions of the values of total leaf area divided by total plant weight, and is a measure of leafiness.

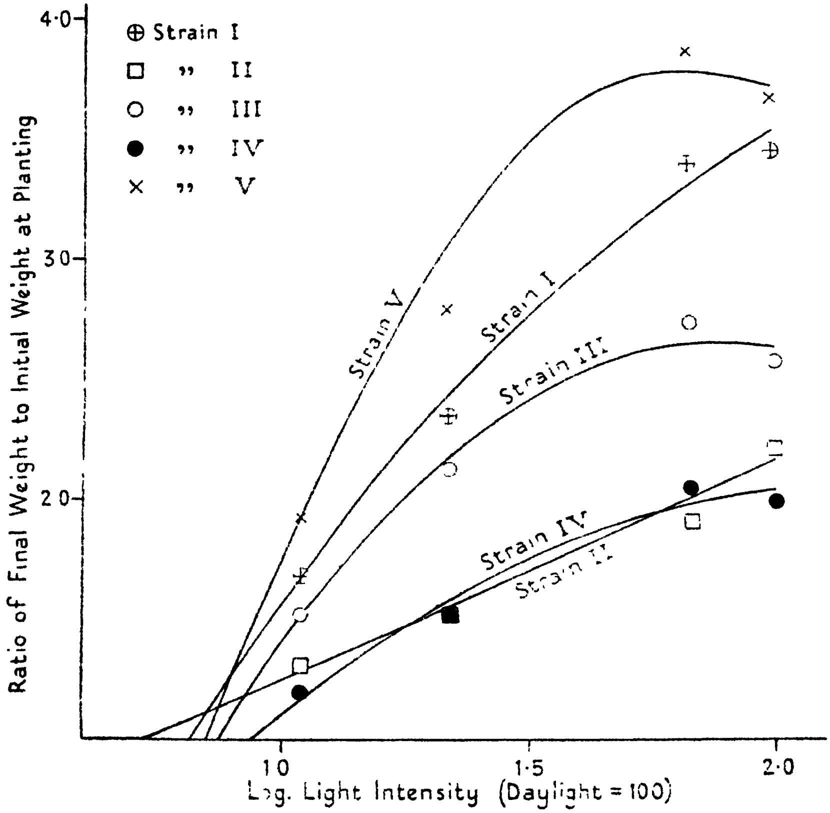

FIG. 2. Experiment XIX. The effects of varying light intensity on the seasonal growth of different strains of *Scilla non-scripta*: the observed means and fitted regressions are both given.

The relationships between strain, light intensity, net assimilation rate, and leaf-area ratio are shown in Fig. 3 *a* and *b*. In a previous paper (Blackman and Rutter, 1948) it was demonstrated that the net assimilation rate is linearly related to the logarithm of the light intensity, and statistical analysis shows that this relationship is not significantly different for the three strains. It is, however, clear from Fig. 3 *b* that there are significant differences in the leaf-area ratios of the three strains. It is also to be observed that for each strain there is a linear relationship between the ratio and the logarithm of light intensity.

Since relative growth rate is the product of net assimilation rate and leaf-area ratio, it follows that the differences in the growth rates of the strains shown in Table XII can be ascribed to the differences in the leaf-area ratios set out in Fig. 3 *b*, i.e. the greater leafiness of strain I as compared to strains II and IV.

A closer examination of these differences in leaf production reveals that the variation is linked with the date of leaf emergence in the early spring. By the time the plots were first sampled and shaded on April 23, strain I had a much larger relative leaf area than strain II, while strain IV was intermediate (see Table XIII). It is, however, apparent that by May 29 the differences between strains are less marked. In fact, there is now a significant interaction between strain and light intensity, for, whereas in full daylight strain II tended to be more leafy than the other strains, at the lower light intensities strain I is more leafy than strains II and IV.

TABLE XIII

Experiment V, 1940. Variations in Leaf Production by Strains and the Effects of Varying Light Intensity

	Leaf-area ratio (cm.^{2} leaf per g. total plant weight).		
	Strain I.	Strain IV.	Strain II.
Before shading (*April 23*)	40·6	35·9	29·6
Log. scale	0·604	0·546	0·460
Significant difference (P = 0·05) = 0·036			
After shading from April 23 to May 28			
Light intensity			
1·0 daylight	24·2	23·7	25·3
Log. scale	0·380	0·373	0·400
0·68 daylight	30·4	27·7	28·4
Log. scale	0·482	0·442	0·450
0·22 daylight	52·9	44·1	47·2
Log. scale	0·722	0·642	0·671
Significant difference (log. scale P = 0·05) = 0·030			

IV. *The Seasonal Growth of Bluebell Plants subjected in the Previous Year to Different Levels of Light Intensity and Mineral Nutrient Supply*

So far, in this series of investigations, attention has been confined to the direct effects of varying light intensity and nutrient supply on the growth of *S. non-scripta* and there yet remains for consideration whether there may be delayed effects of such treatments which only show themselves in the subsequent season. To investigate these possible after effects, mature dormant bulbs were taken from two experiments (for further details see Blackman and Rutter, 1947, expt. III, 1938, and expt. IV, 1939) in which there were 24 treatments made up of 3 light intensities and 8 levels of mineral nutrient supply, namely, C, N, P, K, NP, NK, PK, NPK. Bulbs from each of the 24 treatments of light intensity and nutrient supply level, after being lifted and weighed in the summer, were replanted in the autumn of the same year and their seasonal growth under the conditions of full daylight was measured in the following spring.

In expt. XX, 15 weighed bulbs, taken from each of the 24 treatments of expt. III, were grown in 1939. Each lot of 15 was divided into 3 replicates of 5 bulbs each, and by confounding the interaction between nitrogen, phosphorus, and potassium with block difference, the 72 plots were planted in 6 blocks. The 24 combinations in each replicate will be referred to as pre-treatments.

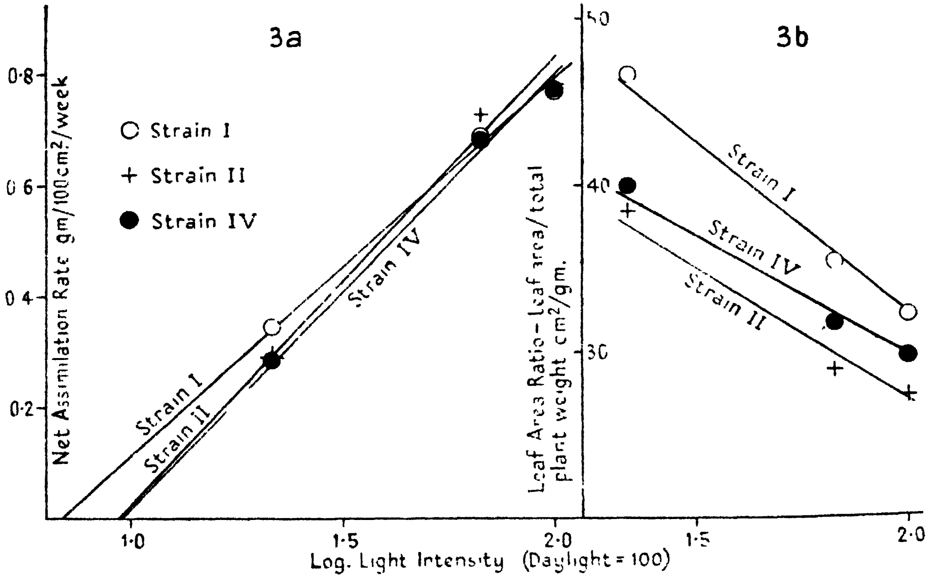

FIG. 3. Experiment V. The effects of varying light intensity on (*a*) the net assimilation rate, and (*b*) the leaf-area ratio of three strains of *Scilla non-scripta*: the observed means and fitted regressions are both given.

There was inevitably a considerable variation in initial bulb size, due to the changes in bulb size produced by the previous light treatments. In consequence, in order to test the significance of the pre-treatment effects, it was necessary to carry out an analysis of covariance, correcting the growth made on to a standard initial weight. After correction the difference between the initial weight and the weight at the time of sampling has been selected on statistical grounds as a measure of growth, since this quantity is statistically independent of initial weights. In order to produce a more normal distribution of the variables, a logarithmic transformation has been employed.

The plots were first sampled on May 16 when 3 plants were collected, while the remaining 2 plants—or rather bulbs—were dug up on August 2, when the leaves had died back and growth had ceased. After correcting for differences in initial bulb size, the statistical analysis showed that there was a significant effect of pre-treatment at the first sampling occasion, in that plants which had been subjected to a low light intensity in the previous year had grown faster than those exposed to full daylight. However, a similar analysis of the data from the sample of August 2 showed no such significant effects.

The changes in weight relative to the mean initial weight of one sample are shown in Fig. 4 for the two sampling occasions. From these results it is concluded that, while the growth made during the whole season is unaffected by the light intensity pre-treatment, a greater proportion of the growth is made early in the season by the plants which had been most heavily shaded in the previous year. Thus, on May 10, plants receiving 0·2 daylight in 1938 had reached their maximum dry weight, whereas those from the full daylight pre-treatment had yet to make a considerable amount of growth.

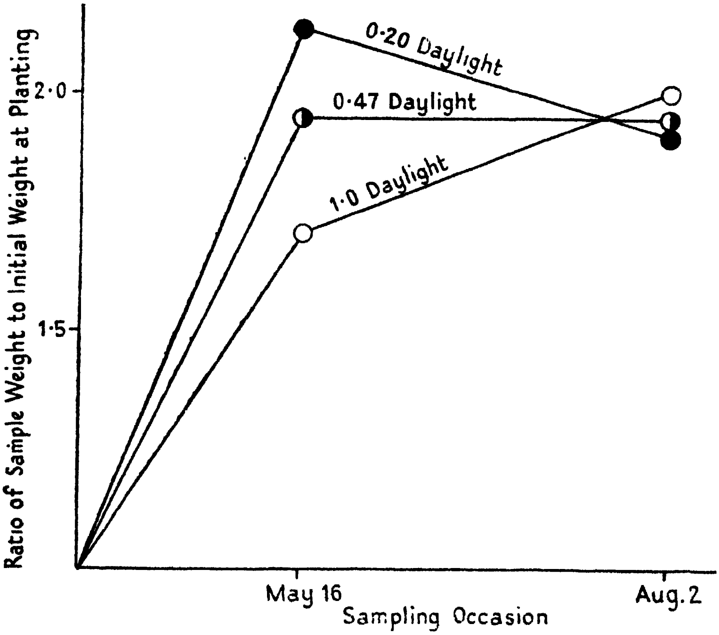

FIG. 4. Experiment XX. The effects of varying light intensity in one season on the growth of *Scilla non-scripta* in the following season when all plants received full daylight. The ratios of sample weight in the second season to initial weight in the previous autumn have been corrected for differences in bulb size.

In seeking for an explanation of these effects it seemed possible that the earlier and faster growth of the pre-shaded plants might be due to the earlier emergence of the leaves and a larger leaf area. The proportion of leaf weight to total weight was therefore calculated for each plot from the data of the first sampling occasion. However, the means of the plants subjected to the three light pre-treatments of 1·00, 0·47, and 0·20 daylight were very similar, namely 0·141, 0·140, and 0·142 respectively. Unless it is postulated that the relation of leaf area to leaf weight had been altered by the pre-treatment, the differences in growth rate cannot readily be accounted for in terms of increased leafiness.

In 1940 a similar experiment (expt. XXI) was carried out with bulbs drawn from expt. IV, 1939. Samples were taken on May 3, when the leaves had reached maximum development, and again on June 4. Measurements of leaf area were made on each date so that any differences in growth rate could be analysed in terms of net assimilation rate and relative leaf area.

When the results from the June 4 sample are corrected for differences in initial weight, the analysis of covariance reveals that there are no significant differences between the three light pre-treatments. The mean weights at this sampling occasion corrected on to the mean initial weight are shown in Table XIV.

TABLE XIV

Experiment XXI, 1940. The Residual Effects of Variation in Light Intensity in One Season on Growth in the Subsequent Season

Dry weight of plant (g.)			
October 1939.	June 4, 1940. Intensity of light pre-treatment (full daylight = 1·00).		
	1·00	0·59	0·22
1·507	3·099	3·587	3·323

Thus if there had been an effect similar to that in the preceding year, i.e. a higher growth rate at the beginning of the season, this sample was taken too late to show it. The earlier sample of May 3 is not suitable for comparison with the initial weight of the previous autumn because over this period there had been a net loss in weight due in part to respiration losses during the winter (Blackman and Rutter, 1947) and the sloughing off of the depleted bulb scales in the spring at the time of leaf emergence. However, an analysis of covariance applied to the increase in weight over the period May 3 to June 4 also revealed no significant effect of light pre-treatment.

In neither of these experiments was there any evidence that differences in the levels of nutrient supply in the previous year significantly affected the growth rate in the subsequent season.

The data of expts. XX and XXI also provide information on the general relationship between rate of growth and bulb size. If x is the initial weight and y the increase in weight, then it may be assumed that the regression relating to y and x passes through the origin if the relationship is linear, but will not do so if the relationship is curved. But if when a linear regression of the form $Y = a+bx$ is fitted to the data, a does not differ significantly from 0, the relation between y and x will also be linear.

In order to attain more normal distributions, the analyses of covariance have been carried out on $\log y$ and $\log x$, so the test of linearity is not whether $a = 0$ in $Y = a+bx$, but whether $b_1 = 1$ in $\log Y = a_1+b_1 \log x$.

The regressions for the two sampling occasions of May 16 and August 2 of expt. XX and the June 4 sampling date of expt. XXI are set out below:

Experiment XX

May 16: $\log Y = 0{\cdot}040 + (0{\cdot}834 \pm 0{\cdot}109)\log x$ (i)

Aug. 2: $\log Y = 0{\cdot}222 + (0{\cdot}543 \pm 0{\cdot}123)\log x$ (ii)

Experiment XXI

June 4: $\log Y = 0{\cdot}302 + (0{\cdot}813 \pm 0{\cdot}102)\log x$ (iii)

Only in the case of equation (ii) does the coefficient of log x differ significantly from 1. Hence it is concluded that, over the period from the autumn until early June, the dry weight increase is linearly related to the initial bulb weight. But when the whole of the annual growth cycle is considered, the growth increment relative to initial weight becomes less as bulb size increases.

Two alternative explanations can be put forward to account for these differences. Firstly, that the ratio of inflorescence size and seed production to total plant weight are higher in large bulbs, so that in the ripening phase, proportionately less material is transferred to the bulb. Secondly, that the leaves of the smaller bulbs persist for longer periods.

DISCUSSION

This is the last paper in this series which will be especially concerned with the autecology of *Scilla non-scripta*, and a discussion of the factors which determine the distribution in different communities must inevitably demand frequent reference to the earlier papers. For the purposes of clarity, the references may cover more detail than normal. In addition, it is proposed to include in the discussion the results of some experiments or observations which have not been previously described, either because they did not conveniently fall into the various sections or are not extensive enough to be considered as separate sections.

Taking first the factors which limit the distribution of the bluebell in grassland communities, it seems clear that the dominant species of grasses in the sward, the factors which control their rate of growth in the spring, and incidence and the intensity of grazing and trampling are all concerned. On the evidence of expts. XI–XVIII it is concluded that the bluebell cannot be a component of the sward in communities which carry a high density of grazing animals from early in the spring. On the basis of expts. XIV–XVIII it is not, however, possible to separate precisely the relative importance of defoliation and trampling. To carry out a precise assessment of defoliation alone would involve many complex and costly experiments to test how far the leaves of the bluebell relative to those of different grasses were palatable or unpalatable to different types of grazing animal. It has, however, been observed that bluebells planted experimentally in woodland away from other vegetation may be heavily grazed by rabbits, but this does not imply that in a mixed sward there would not be a preference by rabbits for other species: indeed, observation suggests that this is so.

With a high density of grazing animals, such as sheep or cattle, the intensity of trampling will still be a major factor even if little defoliation of *S. non-scripta* takes place. The period and duration of the trampling must, however, be taken into account since it is apparent from expts. XVII and XVIII (Tables X and XI) that a combination of frequent and early trampling in the spring is the most injurious.

These biotic factors will in turn be dependent on the type of grassland community, the type of grazing animal, and the management of grazing by man. It has been shown that the growth of grassland in the spring is controlled by rising temperature, nitrogen supply, and the dominant species in the sward (Blackman, 1936; Jones, 1933*a*, 1933*b*). Growth commences earliest where there is a high available nitrogen status in the soil and the dominant species consists of early grasses, such as *Lolium perenne*. In fact, in southern England, stock will be turned into such grassland at a time when the bluebell is just beginning to make active growth in April (see Blackman and Rutter, 1947). Thus the liability to injury from trampling will be maximal under such conditions.

On the other hand, the spring growth of the sward will be delayed if there is a lack of available nitrogen in the soil and the dominant species consist of the types which start growth later in the season because they demand a higher level of temperature for active growth, e.g. *Agrostis* spp., *Holcus* spp., *Festuca* spp. In consequence, if stock are not permitted to graze the sward until considerable growth has been made, then under such conditions of a late spring growth delay in the incidence of trampling will favour *S. non-scripta*.

There are, however, major differences in the control of the grazing exercised in these early or late types of grassland. In the latter, the control of grazing is minimal and often is little different from the free-range grazing. Here the density of stock is limited by the food available during the winter months, so that the number of animals per unit area is not high. Moreover, as soon as active growth starts in the spring, the grassland will be understocked, there will be less movement of the animals in search of food, and the intensity of trampling will be slight. There will, however, be a critical period in the early spring between the emergence of the bluebell leaves and the rapid growth of the grasses when the sward will be grazed hard and more heavily trampled.

Coupled with the effects of trampling and defoliation, there is the direct competition between *S. non-scripta* and the grasses. From expts. XI–XIII (Tables I–V) it is clear that the competitive conditions which are most inimical to the growth of the bluebell are those in which the grasses are not grazed and have received additional nitrogen. In contrast, on the evidence of expt. XI (Table I) the most favourable conditions will occur where there is a high level of nitrogen supply and only the grasses are grazed without any attendant heavy trampling. Conceivably, such selective grazing could occur with rabbits, but would certainly not result from grazing by larger herbivores. Again, a high availability of nitrogen in the spring implies a fertile soil and these conditions in turn will favour the dominance of early grasses like *L. perenne*. In consequence, such grassland will be the first to be grazed in the spring by stock—a set of conditions which will be wholly inimical to the bluebell. Conversely, if the sward is not grazed, it is apparent from Table I that the adverse indirect effect of a high nitrogen level in increasing the growth and thereby the shade cast by the grasses more than outweighs the advantages of increased nitrogen supply to *S. non-scripta*.

If shading is a major factor in the competition between the bluebell and grasses, then it would be expected that seedlings or very small plants of *S. non-scripta* would be more adversely affected than larger plants, and this is supported by the results of expts. XII and XIII. It would also be expected that the degree of shading would be greater in the case of tall grasses, provided that no other major factor is involved. The fact that the smallest bluebell plants growing in competition with *F. rubra* made less seasonal growth than those competing with the taller *D. glomerata* (see Tables IV and V) suggests that other factors may also be operative.

There is also evidence that the degree of competition varies with the season. In the case of the comparable experiments for 1939 and 1940, where *F. rubra* was in competition with the smallest plants, in 1939 the seasonal gain in weight was 99 per cent. in the uncut sward receiving nitrogen and 132 per cent. on the control, while for the comparable treatments in 1940 the plants lost weight (Table V). It is considered that this seasonal variation is due to the differences in spring rainfall; 1940 was a particularly dry year and 1939 a wet one. That in 1940 water availability was a more important factor in limiting growth than in the previous years is confirmed by the experiments on light intensity and mineral nutrient supply (Blackman and Rutter, 1947, expts. III, IV, V, VII, VIII).

In considering therefore the interrelationship between the biotic factors and those concerned with direct competition with the grasses, it appears that the following set of conditions would be most favourable to *S. non-scripta*: (i) the soils should be of a low mineral nutrient status; (ii) the dominant grasses should possess a prostrate habit; (iii) the grasses should not start active growth until late in spring; and (iv) the sward must not be grazed until after the bluebell plants have flowered. The fact that *S. non-scripta* is more frequently found outside woodland in Scotland, in Wales, and in the south-west and north of England is therefore significant. It is in these regions that the soils tend to be leached. It is here that grassland communities dominated by such grasses as *Agrostis*, *Festuca*, and *Holcus* species are of more frequent occurrence. It is here that there is a higher proportion of rough or hill grazings which in turn provide sites where there is either no grazing at all because of the steepness of the slopes or where, at the higher altitudes, grazing in the early spring does not take place because the flocks have not been moved up from wintering in the valleys.

Although it is clear that these favourable conditions are found less frequently in the south and east of England, it must be emphasized that there is a complicating factor, namely, that in the west and north of England there is a higher rainfall. In fact, for the drier areas there is some data which suggests that even with the most favourable combination of the factors already defined, the conditions in grassland are rarely advantageous to *S. non-scripta.*

This evidence is forthcoming from an experiment in which bulbs of flowering size were planted in an infertile gravel soil near Slough where the vegetation had not been grazed for several years, but had from time to time been cut in the summer. In consequence, the dominant grasses consisted of *F. ovina* and *Arrhenatherum elatius*, together with *Hypericum perforatum*, *Centaurea nigra*, and *Lathyrus pratensis.* After the dominant bulbs were planted in the autumn of 1937, they were left for 4 years and dug up again in July 1941, when they were in the dormant phase. During this period the following series of treatments were applied. In one series the herbage on the plots was left to grow without any interference throughout the year, but some of the plots received each year in the early spring either additional nitrogen (50 lb. N per acre) or phosphorus (75 lb. P per acre) or potassium (75 lb. K per acre). In another series the plots were defoliated once either before the flowering of the bluebell or at a time when the plants were in flower. In the third series the plots were *permanently* covered with screens either of butter-muslin or perforated zinc for the whole of the four years.

TABLE XV

Experiment XXII. The Effects of Additional Nutrients, Defoliation, and Shading over a Period of Four Years on the Growth of S. non-scripta *in a* Festuca-Arrhenatherum *Sward*

Treatments.	Final weight (log. wt. of 10 bulbs).	Ratio of final to initial weight.
Series I. No defoliation of sward		
(i) Control	0·965	0·87
(ii) Nitrogen added to sward . .	0·974	0·91
(iii) Phosphorus added to sward. .	0·964	0·89
(iv) Potassium added to sward . .	0·963	0·90
Series II. Sward defoliated		
(i) Before flowering of bluebell . .	0·749	0·57
(ii) When bluebell is in flower . .	0·878	0·69
Series III. Sward shaded		
Intensity of shade 0·5–0·6 daylight .	1·289	1·64
„ „ „ 0·2 „ .	1·199	1·80
Sig. diff. between treatments ($P = 0{\cdot}05$)	0·129	

From Table XV it is evident that in the ungrazed sward, irrespective of the level of nutrient supply, there has been over the 4 years a decrease in weight of approximately 10 per cent. Moreover, the reduction has been increased to 30 per cent. even when a single defoliation has taken place as late as the full flowering stage. Since these results have been obtained with flowering plants and in view of the evidence that small plants are less competitive than large plants, it is concluded that at least in the drier areas, *S. non-scripta* will seldom invade grassland communities through seed dispersal, but that mature plants may survive for long periods where woodland has been cleared and there is no or very little grazing in the spring.

Perhaps the most interesting results in Table XV are those for Series III, where shading has clearly shifted the balance of competition in favour of *S. non-scripta.* This shift could be explained on the basis that the bluebell is better adapted to conditions of low light intensity than the other components of the community. Before, however, considering the available evidence for such a conclusion it must be emphasized that the figures given for the degree of shading, namely 0·5–0·6 and 0·2 of daylight, are somewhat misleading. During the spring the percentage of light transmitted by the screens were as stated, but later in the season the figures are too high. In order to minimize the proportion of lateral light reaching the plants under the screens they were placed in position some 12 in. above the ground. As a result, by midsummer, the tops of the shoots of the grasses and other species reached and pressed against the surface of the screens, so that the lower halves of the plants were much more heavily shaded. Moreover, the effect was greatest in the first year, since the initial growth made in the shade will in part be due to the utilization of reserves in the leaf bases and roots (Blackman and Templeman, 1938).

The experimental shading therefore had the effect of increasing the degree of shading in the summer months below 0·5 and 0·2 daylight. Thus the conditions were such that the shading was more intense for the grasses and other plants whose seasonal growth extended from the spring to the autumn than it was for *S. non-scripta* with a growth cycle confined to the spring months. In other words, the light conditions were not unlike those of deciduous woodland.

As to whether the light requirements of *Festuca* and *Arrhenatherum* and the other species are greater than those of the bluebell, there is no direct evidence. There is, however, indirect evidence from the results of investigations, which will shortly be published in this series, that in comparison with some ten other species of plants the reactions of the bluebell to low light intensities are by no means exceptional.

Turning next to a consideration of woodland conditions, all the evidence of the previous series of investigations emphasizes that the environment is favourable to *S. non-scripta*, not because of the shade but because in woodland heavy grazing animals are absent and therefore adverse biotic factors do not operate.

However, in woods which are much frequented by man the trampling factor is not entirely absent. For example, in the woodland investigations it was observed that even in a single season the paths taken to the experimental areas could be picked out by the bruised leaves. Such trampling will certainly occur where many people come to pick the flowers, and it is this trampling rather than the picking which will be injurious. There can be no possibility that the actual plucking of the flower-stalk is damaging to the bud initials, since periodic dissection of samples of the bulbs has shown that the primordia of next year's leaves and inflorescence have not been formed.

In a recent paper Peace and Gilmour (1949) have reached the same conclusions that neither picking the flowers nor pulling the flower-stalk has any deleterious effect on the number of flowers produced in subsequent seasons. They too concluded that the trampling and bruising of the leaves associated with picking could lead to a considerable reduction of flowering.

It is of some interest to note that in one of their woodland experiments there was some indication that removal of the inflorescence increased the number of flowers in the following years. Such an increase would be expected on the basis of the previous investigations (Blackman and Rutter, 1947) since there will no longer be a partition of the assimilates between the developing inflorescence and the bulb, and this will lead to larger bulbs and larger bulbs will in turn tend to split and produce offsets and hence more inflorescences in the following seasons.

In fact, under conditions which favour vegetative reproduction rather than dispersal by seed, picking the flowers, as long as the effects of trampling are minimal, may be advantageous. The removal of the inflorescences is not confined to man's activities, for it has been observed on several occasions in woodland that the flower-stalks have been bitten off (probably by rabbits) at the time of flowering.

In the majority of woods the biotic factors so far instanced can be regarded as unimportant, but the possibility that the distribution of the bluebell may be related to the amount of fallen leaves has yet to be considered. It is self-evident that the annual deposition of leaf litter must bear a relationship to the denseness of the overhead canopy and this in turn will be related to the degree of shading. The direct relationship established in the woodland investigations between bluebell density and the degree of shading might therefore be interpreted in terms of variations of litter accumulation. The evidence of the woodland investigations (Blackman and Rutter, 1946) does not support this view. In two of the woods, a *Larix japonica* plantation and a beechwood, the marked gradient in light intensity on the experimental areas was due rather to 'side light' entering from the edge of the wood than to variations in the overhead shade. In consequence the correlation between the degree of shading and leaf deposition was small. Again, in the third wood, where the density of the bluebells under holly-trees and a canopy of mixed ash and beech was related to variations in light intensity, statistical analysis did not indicate that the differences in litter deposition or the type of litter were significant factors.

The possibility that the type of litter and rate of breakdown might affect the supply of nutrients has already been discussed in the 1947 and 1949 papers, and it was concluded that for established plants light rather than nutrient supply is the controlling factor in closed woodland. On the other hand, where there is a slow rate of breakdown and a dry surface layer of undecomposed leaves, the conditions are less favourable for the establishment of seedlings.

In considering the pre-eminence of the light factor and the distribution of *S. non-scripta* in these woodland communities, it should be emphasized that where other plants were present in the ground flora their density was not great and they were of a prostrate habit and did not compete for light. Such conditions do not always hold; for example, where *Mercurialis perennis* is also present, *S. non-scripta* is generally not found if the density of the shoots of dog's mercury is high and their growth is luxuriant.

In open woodland, where the level of light intensity during the summer months is higher than in closed woodland and plants other than those with a spring growth cycle can therefore grow successfully, competition between the bluebell and other components of the ground flora may be accentuated. For example, grasses such as *Holcus mollis* may occur, while taller plants such as *Rubus* spp. and *Pteridium aquilinum* will also be present.

On the basis of the present series of investigations it is of some interest to re-examine the conclusions reached by Woodhead (1904, 1906) on the inter-relationship between *S. non-scripta*, *H. mollis*, and *P. aquilinum* in some Pennine oak woods. In the deepest shade the bluebell is found growing alone, in areas of intermediate shade all three plants occur together, while in the most open sites where *P. aquilinum* makes luxuriant growth it tends to become dominant. It is not considered that the presence of the bluebell in the areas of lowest light intensity can be ascribed to its greater ability to withstand shade, for it has already been shown that the compensation point for *S. non-scripta* is approximately 0·09 of daylight (Blackman and Rutter, 1948), while Salisbury (1918) concluded that bracken was not found in the oak hornbeam woods where the light intensity fell below 0·04 of daylight in midsummer and did not form a closed society below a minimum intensity of 0·11 daylight. From the previous studies of the seasonal fall in light intensity of different woodlands (Blackman and Rutter, 1946) it is evident that a light intensity as high as 0·11 daylight in midsummer is only found in open woodland. Secondly, considering the lower limit for bracken of 0·04 daylight, this mid-season intensity will correspond to light intensities in the early spring of 0·6 daylight or more, according to the type of woodland.

The contrast does not therefore lie in the varying reactions to deep shade of the two species but in the difference between the species as to the time of the emergence of the shoots and commencement of active assimilation. The bluebell will have made approximately half or a third of its seasonal growth by the time leaf expansion of the oak-trees is taking place, while the expansion of the bracken fronds will not be complete before the oak canopy has fully developed. Where *S. non-scripta* is growing alone in closed woodland, that is where the *minimum* mean spring light intensity is of the order of 0·05 daylight, the total seasonal growth made will be compounded of the gain made in the high-light phase, and the loss during the low-light phase when the degree of shade will be below the compensation point. Where bracken is also present, i.e. in the less shaded areas, though there will be some advantage to *S. non-scripta* in the increased light intensity during the high-light phase, this will be offset later by the deeper shade in the low-light phase. Then the shading will in part be due to the canopy and in part due to the bracken, and the greater the height and density of the fronds, the lower will be the light intensity. It is considered that it is this direct shading by dense bracken which is operative in the suppression of *S. non-scripta*.

In the case of bluebell seedlings, apart from the light factor, it is probable that where the accumulation of bracken litter is pronounced, the conditions are unfavourable for establishment.

Turning next to the association between *S. non-scripta* and *H. mollis*, the essential criteria for the successful establishment of bluebells in grassland will also hold. There will be no grazing, *S. non-scripta* will start growth well in advance of *H. mollis*, and the rainfall in the Pennines is relatively high. The main difference between the woodland and grassland environments will be that at all times in woodland there will be some overhead shade, but the degree of shading will be greater for *H. mollis* than for *S. non-scripta* since with its longer and later period of growth the grass will be more subject to shading by the bracken and the trees. In fact, the conditions will not be unlike those of expt. XXII, where the grassland plots were permanently shaded and 'self-shading' became progressively greater as the season advanced. From the aspect of the bluebell, the tree shade during the high-light phase will restrict growth more than in grassland. This disadvantage will, however, be offset by the effects of mid-season shade in decreasing the growth of *Holcus*, so that there will be less active growth in the spring and therefore less competition with the bluebell.

In many of the damper oak woods it has been observed that where the canopy is not continuous or the shrub layer is not well developed, *Rubus* spp. rather than *P. aquilinum* are often dominant and in such areas *S. non-scripta* is absent. This pattern of distribution, it is considered, can again be interpreted in terms of the light factor. Even during the high-light phase, the bramble shoots cause a marked reduction in light intensity at ground level and this is further accentuated when new foliage is produced. Such conditions are akin to those in evergreen woodland where, with the absence of an initial high-light phase, the mean degree of shade is in general inimical to the bluebell (Blackman and Rutter, 1946).

Although the vernal fall in light intensity in deciduous woodland contrasts most with the small decrease in evergreen woodland, there is experimental evidence that the mean light intensity during the period of active growth is more important than the overall fall in intensity between the high- and the low-light phase. For example, at the Field Station, Slough, dormant bulbs of known weight were planted in groups of 10 in a number of sites so selected that the shade was provided either by evergreen plants (e.g. *Pinus sylvestris*, *Viburnum tinus*) or by deciduous trees (e.g. *Betula alba*, *Corylus avellana*, *Sambucus nigra*). Planting took place in the autumn of 1937, and in the following spring the degree of shade was measured at weekly intervals for each site until June, when the bulbs were lifted and reweighed.

From Fig. 5 it is seen that the change in weight, expressed as the ratio of the final to the initial weight in the previous autumn, largely follows a logarithmic relationship with mean light intensity, irrespective of whether the fluctuations of light intensity about the mean are large (deciduous overhead canopy) or small (evergreen canopy). It should also be noted that below 0·09 daylight there is a net loss in weight and that at the light intensities of 0·05 and 0·014 of daylight the losses over the yearly cycle amount to 34 and 66 per cent. respectively. It is therefore clear that even plants with large bulbs cannot persist for long in deep shade.

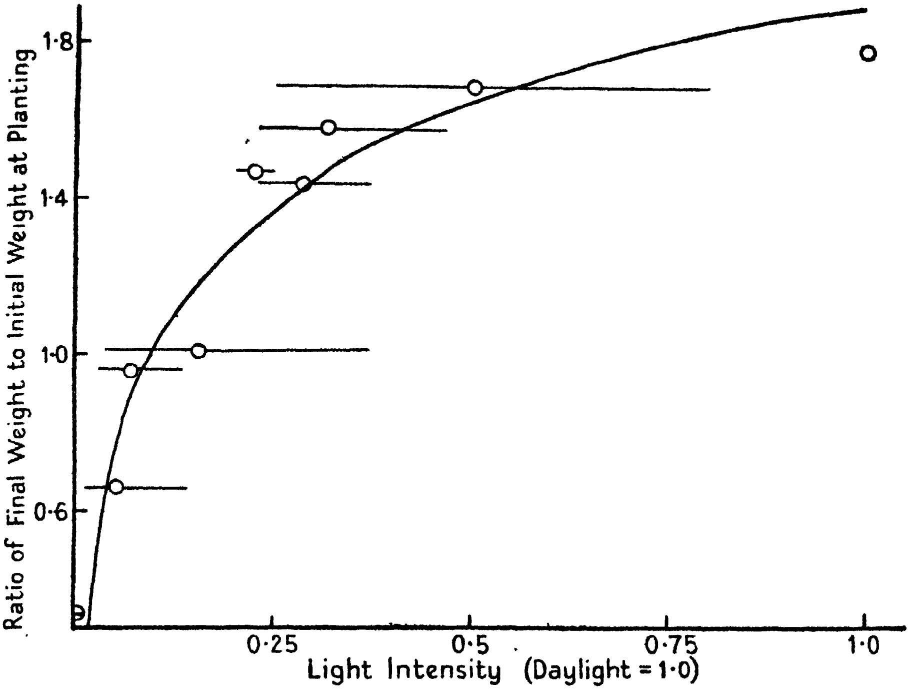

FIG. 5. Experiment XXIII. The variation in the seasonal growth of *Scilla non-scripta* subjected to shading by different trees and shrubs. Besides the mean light values during the spring (end of March to June) for the nine experimental sites, the minimum and maximum degree of shading has also been indicated for each site.

Although it is evident from Fig. 5 that over the wide range of light intensity from 1·0 to 0·01 of daylight the mean light intensity rather than the fluctuations about the mean control the seasonal growth, it does not follow that within a narrow range this is also true. It has been shown previously (Blackman and Rutter, 1948) that where plants have been grown in full daylight until the beginning of May and have been subsequently shaded they make less growth than plants that have been constantly shaded at a comparable mean light intensity for the whole period. It was also demonstrated that this difference was due to the greater assimilating area of the constantly shaded plants.

This aspect of the light factor must be taken into account in considering the association of *S. non-scripta* with bracken in sites where there is no overhead canopy. In the high-light phase before the expansion of the bracken fronds, the net assimilation rate will be higher than in the woodland sites, but the leaf area will be less. The differences therefore in the growth made within the two light environments will be dependent on the balance between assimilation and leaf area and these, in turn, will be dependent on the differences both in the length of the high- and low-light phases and in the relative light intensities operating over the two periods.

In deciduous woodland under conditions which permit of a close stand of bracken, the highest light intensity will be of the order of half to three-quarters of daylight, but the intensity will fall rapidly as the tree leaves expand and this will normally take place before the expansion of the bracken fronds is complete. On the other hand, in the open, once the bluebell leaves have emerged through the litter, they will be receiving full daylight and the high-light phase will persist for longer than in woodland. However, in the low-light phase, especially where there is a very vigorous growth of bracken in the open situation, the degree of shading will be of the same order under both sets of conditions, i.e. generally below 0·05 of daylight.

On the basis of these considerations and the results of the earlier investigations, it is concluded that in the open sites the effects of length and intensity of the high-light phase in raising the net assimilation rate will more than counterbalance the larger leaf area of plants growing in woodland. In consequence, the seasonal growth will be greater. From general observation of sites outside woodland it is clear that the bluebell will persist in tall and dense stands of bracken. For example, in Bagley Wood, near Oxford, *S. non-scripta* can still be found in an ungrazed area which was cleared of trees over 30 years ago and where the bracken now reaches a height of 2 metres.

This equilibrium between the bluebell and dense bracken is not only related to the light factor. Because of the deep shade cast by a dense stand of *P. aquilinum*, plants such as the grasses, which have an extended growing season, are suppressed and competition between the bluebell and other plants during the spring will be restricted to those which also make their active growth in the high-light phase. Such a limitation of competing species may well be more important in areas of low rainfall.

The establishment of dense stands of bracken implies that at least during the summer months heavy grazing animals have not been present in any numbers on the area, since the consequent trampling will be inimical to the formation of such closed communities. The association of bracken and bluebells found on steep slopes in the wetter parts of Great Britain will therefore in part be dependent upon their inaccessibility to grazing animals. On the other hand, slopes in less humid areas, where the soil dries out rapidly, will be unfavourable habitats for *S. non-scripta*.

In considering the occurrence of bluebells on slopes in areas of high rainfall another factor must be taken into account. Under such conditions there will be free drainage and no possibility of a temporary waterlogging of the soil. Although general observation indicates that *S. non-scripta* will grow under relatively wet conditions, there is evidence that it will not tolerate waterlogged soils.

For example, in the Japanese larch plantations at Shurlock Row, Berks., where the relation between bluebell density and light intensity was investigated (Blackman and Rutter, 1946), it was observed that in one area of relatively light shade bluebells were singularly absent except on the raised banks of a ditch. In order to investigate experimentally the growth of bluebells in this area, 60 graded dormant bulbs of known weight were planted in the autumn along transects running inwards at right angles to the edge of the plantation. During the latter half of the subsequent winter this area was waterlogged and a high proportion of the planted bulbs failed to emerge above ground in the spring. On investigation it was found that this failure was due to the rotting of the bulbs by *Penicillium cyclopeum*. That the attack of this fungus is favoured by a high soil moisture content has also been found by Singh (1941). It is not, however, inferred that attack by this mould is confined to such conditions, since during the collection of dormant bulbs from several woods it has been observed that a number of the bulbs possessed necrotic lesions. On the other hand, in other sites when bulbs were dug up during the spring at periodic intervals, serious damage has not been noted.

Lastly, in considering the main factors responsible for the distribution of *S. non-scripta* there remains the question of the importance of soil types, and here it is not so much a matter of enumerating the most favourable soils but of defining the soil conditions where the bluebell is absent. Watt (1934) points out that on the Chiltern plateau beech woods *S. non-scripta* may be abundant on patches of sand, but is rare or absent on adjacent areas of clay with flints. In beech woods of the Berkshire Downs it has been observed that where the chalk is very close to the surface the bluebell disappears. Again, well-defined podzols are in general unfavourable habitats, but bluebells will grow under less acid conditions, i.e. soils showing weak podzolization but with a mull surface layer.

The experimental results of the 1947, 1948, and 1949 papers in no way indicate that variations in the nutrient status of different soils are important. On an infertile gravel soil where the growth rate of other plants was greatly increased by the additions of nitrogen, phosphorus, and potassium, the gains made by the bluebell were small and in an oak wood negligible. Again, since *S. non-scripta* even in full daylight only doubles or trebles its weight during the year, it is unlikely that there can be a demand for a high nutrient level in the soil, and in woodland with the consequent slower rate of annual increase the need will be even less.

Since it is on the lighter soils, in contrast to some heavy clays, that the bluebell is prevalent, the difference may be in the physical characteristics. As Chouard (1926) has observed, bluebell seedlings at an early stage start to form a bulb and produce contractile roots, and with the years there is a progressive movement downwards to a maximum depth depending on the soil type. Since a bluebell seed only weighs 0·005 g. and since at 0·2 daylight bulbs will only double their weight in the season, this process is inevitably slow. It is estimated that in woodland by the time bulbs reach their maximum depth they will be at least 7 to 10 years old.

Therefore during the period of establishment the plant will remain in the surface layers, which in dry areas or dry springs will be most prone to dry out. If clay soils prevent or slow down the downward movement of the bulb, then the liability to drought may be increased. Such an explanation could be equally applicable to very shallow calcareous soils where the underlying chalk would offer a physical barrier to further downward movement.

As an alternative explanation it could be put forward that the depth to which bulbs penetrate is determined by the carbon dioxide or oxygen tension of the soil atmosphere, and that in clay or very wet soils a steep gradient with depth of oxygen or carbon dioxide concentration, or a combination of both, may confine the bulbs to the surface layers. Certainly, in light soils, the larger bulbs may reach a depth of 20–25 cm., while at the other end of the scale in wet situations the limit may be 10 cm. or less. Such a variation has been observed on a gentle slope terminating at the bottom in marshy ground.

There is perhaps one further possible environmental factor which must be taken into account in assessing the differences within and without woodland habitats. The overhead canopy will give some protection from the effects of spring frosts and wind velocities will be lower than in the open. The available evidence does not, however, indicate that the shelter provided by woodland is important. In the three years when bluebells were planted in the open, no signs of frost injury were observed and the plants made more growth than those which were lightly shaded and therefore to some extent protected. Again, it has been already stated in the introduction that *S. non-scripta* can be found growing on rock ledges and the tops of cliffs exposed to the Atlantic. It is, however, possible that in areas of low rainfall high winds may have an indirect effect by increasing evaporation of water from the surface soil.

Lastly, there remains for discussion the underlying causes for the differences in the growth rates of the five strains of bluebell. From expt. XX there is evidence that variations in the light intensity in one season may affect growth in the subsequent year, and it will be recalled that whereas bulbs of four out of the five strains were collected from woodland sites, the fifth (strain IV) had been grown in the open. In consequence, the 'light histories' in the year previous to expts. V and XIX were very different. Since the soil conditions also differed between sites, it might be advanced that differences in the mineral nutrient reserves between strains might carry over to the subsequent year. Again, in expts. XX and XXI it has been established that the rate of seasonal growth is dependent upon bulb size and, as the initial bulb weights varied between strains, this variable must also be taken into account.

Considering first the hypothesis that the differences between strains are related to variations in the mineral nutrient reserves, the available evidence is against such an assumption. In expts. XX and XXI variations in the level of nitrogen, phosphorus, and potassium in one season in no way influenced growth in the following year. Moreover, in the 1947 and 1949 papers it has been shown that below half-daylight the level of nutrient supply has little effect on the growth rate, while it is clear from Figs. 1 and 2 that the differences between strains persist at 0·22 and 0·11 of daylight.

Taking next the question of how far variations in light intensity between habitats may have influenced the growth rate in the subsequent experimental period, it is considered that the differences between strains are far too large to be accounted for on this basis. For example, in expts. XX and XXI there were no significant differences in the adjusted ratio between the final and initial weights of plants which had been grown under varying light intensities (1·0, 0·59, and 0·22 daylight) in the previous year.

Turning next to how far differences in initial bulb size between strains may have affected the differences in seasonal growth, in several comparisons this question does not arise since the initial bulb weights were very similar. In expt. V, strains I and IV had the same initial weight but yet differed in their growth rates. The results of expt. XIX require more consideration since the initial bulb weights of the five strains ranged between 0·566 and 0·701 g. It is, however, possible to gain some idea from expt. XX of the magnitude of the variations in seasonal growth in terms of the variations in initial bulb size. For plants growing in full daylight it is possible to calculate by means of formula given on page 504 the ratio between final and initial weight; over the limits of 0·55 to 0·79 g. the ratio varies from 1·69 to 1·76 for plants given full daylight. On the other hand, within precisely the same limits of initial bulb size, the variations in the ratios for the five strains are from 1·98 (strain IV) to 3·65 (strain V). In view of these large differences it must be concluded that the differences in the growth rate between strains are inherent characteristics.

The further analysis of the differences between strains I, II, and IV in expt. V (Fig. 3) has shown that the variations in growth rate are independent of the net assimilation rates but dependent both on the relative leaf-area ratios and the time of shoot emergence in the spring (see Table XIII). The importance of the high-light phase in the seasonal growth of the bluebell in different environments needs no further emphasis, and it is clear that an ecotype which produces a large leaf area early in the spring has a potential capacity for successful establishment under a wider range of habitats. On the other hand, it does not appear that ecotypes vary in their ability to survive in deep shade.

In conclusion, it is hoped that this discussion has served to emphasize how great are the advantages of linking general ecological observations with both precise field studies and multifactorial experiments for a proper understanding of the interaction and relative importance of environmental factors. In the case of the bluebell a proper understanding has led to a new interpretation of the factors which limit its distribution both within and without woodland. Further investigations are now required to determine whether this interpretation is also applicable to other woodland plants, especially those species which make active growth in the high-light phase of spring. In view of the fact that several woodland plants can be found growing in the open at high altitudes, it may well be that they too grow best in full daylight and are normally prevalent in woodland because they are there protected from trampling and grazing by herbivores.

There is also a great need for further precise investigations on the growth and development of plants in different habitats. For this purpose the techniques of growth analysis will serve as a valuable link between the field and the laboratory: indeed, on occasion, they may also serve as a useful corrective. The concept of an obligate shade plant rests largely on laboratory investigations in which the assimilation of leaves rather than whole plants has been measured at varying light intensities, and the conclusion drawn that the assimilation rate may reach a maximum at light intensities far below full daylight. The present field investigations have demonstrated that up to full daylight the net assimilation rate of *S. non-scripta* is logarithmically proportional to the light intensity. There is supporting evidence that this relationship also holds for *Geum urbanum* and *Solanum dulcamara.* In fact it will be shown in a later paper that variations in the growth rates of plants under shade conditions are related to differences in the total leaf area, rather than differences in the net assimilation rate.

SUMMARY

In the four previous papers it has already been established, firstly, that *Scilla non-scripta* grows best in full daylight, secondly, that the light intensities ruling in closed deciduous woods are the major factors controlling the growth rate and distribution, and thirdly, that the bluebell does not require a high level of mineral nutrients. Yet, on the other hand, *S. non-scripta* is normally confined to woodland and it is only in restricted localities that this plant occurs outside woodland, more particularly in grassland or bracken (*Pteridium aquilinum*) communities. The present paper is concerned with the further analysis of the factors which may operate in limiting the distribution.

When the bluebells are planted in swards dominated by one of the following grasses—*Phleum pratense*, *Festuca pratensis*, *F. rubra*, or *Dactylis glomerata*—the conditions are most inimical to the growth of *S. non-scripta* when additional nitrogen has been added to the sward early in the spring and the grasses have been allowed to grow unchecked. Conversely, maximum seasonal growth takes place when the shoots of the grasses but *not* those of the bluebell are frequently defoliated, and here adding nitrogen favours the growth of *S. non-scripta.* It is therefore concluded that when the grasses are not cut a high nitrogen level operates adversely by increasing the degree of shade cast by the grasses, while in the absence of shading due to differential defoliation competition for nitrogen comes into operation.

There is experimental evidence that large plants compete more successfully with the grasses than small plants which have not reached the flowering stage.

The intensity of competition is also dependent upon the dominant species in the sward. A comparison of the results for the two years (1938 and 1939) indicate marked seasonal differences. In 1938, a wet spring, small plants more than doubled their weight during the season, while in the following dry year comparable plants lost weight. In another experiment where 'flowering size' bulbs were planted in a *Festuca–Arrhenatherum* sward in 1937 and left for 4 years, the plants barely maintained their original weight over the period.

In an experimental assessment of the possible effects of grazing and trampling it has been found that the removal of the leaves and inflorescence well before the flowers open causes more injury than defoliation at a later stage. A double defoliation before and again during flowering brings about the maximum reduction (40–60 per cent.) in seasonal growth. When a *Festuca–Arrhenatherum* sward containing newly planted bluebells was defoliated once each spring, either at or before the flowering stage of the bluebell, then after a period of 4 years the final bulb weight was 31–43 per cent. less than the weight at planting. The adverse effects of trampling increase with the incidence and frequency. Trampling at intervals of a week significantly diminishes the growth rate more than trampling at 2- to 4-week intervals. Trampling at 10-day intervals from the time of leaf emergence significantly reduces the final weight more than when trampling is initiated 20 days later.

A comparison has been made of the effects of light intensity and mineral nutrient supply on the growth of plants from four different woodland habitats and from one site where the plants had been grown in the open. These samples from the five populations ('strains') showed striking differences in the seasonal growth rate. In full daylight the fastest growing strain gained 85 per cent. more in weight over the season than the slowest. Comparable differences were also found at lower lighter intensities, but the calculated light values at which no gain in weight should take place only varied between strains from 0·05 to 0·087 daylight.

For three strains which differed significantly in relative growth rate (efficiency index) it was found that while the linear regressions of net assimilation rate against the logarithm of light intensity did not differ significantly, yet similar regressions of leaf-area ratio (leaf area/total planting weight) showed marked differences. Apart, however, from these differences in leafiness, causing differences in the growth rate, the strains also varied in the time of shoot emergence in the spring and the commencement of active assimilation. There was no evidence that the strains varied in their response to different levels of nitrogen, phosphorus, and potassium.

With increasing bulb size, the subsequent relative gain in weight during the season decreases. It has been found that when plants are grown in full daylight this relationship can be empirically expressed as $Y = 1{\cdot}66x^{0{\cdot}543}$, where x is the initial bulb weight in the autumn and Y the increase in dry weight at the end of the following summer.

Two experiments in different years were carried out to determine whether plants which had been subject to varying levels of light intensity (0·2–

1·0 daylight) in one year grew in full daylight at different rates in the following year. In one experiment the light pre-treatments had no significant effects; in the other there was evidence that while the growth made during the whole season was unaffected, a greater proportion of the growth was made early in the season by the plants which had been most heavily shaded in the previous year.

On the basis of the present and past investigations, the conclusions reached are that the bluebell will be excluded from habitats where the following factors operate: (i) the mean light intensity between March and June is less than 0·05–0·07 daylight, or (ii) the ground is waterlogged or flooded during the winter, or (iii) the area is grazed or repeatedly trampled by large animals during the spring. The necessary conditions for the successful growth of the bluebell in grassland communities are: (i) the soils should remain moist in the spring but the drainage must be good; (ii) the mineral nutrient status of the soils should be low; (iii) the dominant grasses should combine a relatively prostrate habit with the characteristic of starting growth late in the spring; and (iv) the sward should not be grazed until after the flowering of *S. non-scripta*.

The bluebell will compete with *Pteridium aquilinum* outside woodland because (i) much of the seasonal growth is made in the high-light phase before the bracken fronds expand; (ii) bracken tends to exclude other competitive species; and (iii) dense bracken is associated with the absence of heavy grazing animals.

S. non-scripta is not susceptible to frosts and will grow in exposed situations (e.g. rock ledges) provided that the availability of water is adequate.

In woodland, shading *per se* can only be regarded as an inimical factor; the prevalence of the bluebell in deciduous woods is attributed to the absence of aggressively competitive species and more particularly to the protection provided by such habitats from grazing animals. Finally, it is advanced that precisely the same factors operate in confining other species to woodland.

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Metabolic Systems in the 'Root' of *Brassica napus* L.

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With three Figures in the Text

ABSTRACT

This is a study of the effects of possible intermediary metabolites on the respiration of root tissue from *Brassica napus* using the Warburg micro-manometric technique. It is concluded that ascorbic acid is oxidized by two systems, one of which appears to be a direct oxidase and the other a dehydrogenase. No evidence of peroxidase activity was secured. A substantial fraction of the total respiratory activity was insensitive to cyanide and azide. The biologically important organic acids were oxidized with the production of carbon dioxide. Glutamic and aspartic acids were metabolized with great rapidity, glycine and alanine much more slowly. A scheme integrating these results is outlined and compared with the respiratory systems existing in potato.

INTRODUCTION

IT is stated in the literature that the root of *B. napus* L. does not contain the polyphenol oxidase system; this statement can be confirmed, for the addition of any water-soluble catechol derivative to the respiring tissue slices does not increase the rate of oxygen uptake.

Observations have therefore been made on the respiratory metabolism of this tissue. They are reported in three sections: the first is concerned with ascorbic acid oxidation; the second with catalase and peroxidase systems; while the third deals with the metabolism of certain biologically important compounds. In the final discussion an attempt is made to compare the metabolic processes in slices of the swede root and the potato tuber.

TECHNIQUE

The roots were purchased locally without reference to variety or uniformity of supply. The tissue was cut into slices 0·025 in. thick and these were washed in running aerated tap-water for between 24 and 144 hours before use. Except when specifically stated to the contrary the slices were suspended in 4 ml. of a solution of potassium phosphate of pH 5·5–5·8 at 24° C. in Warburg micro-respirometer flasks. It was found that phthallate buffers had a depressor effect upon the rate of respiration and that other buffers of suitable pH value contained substances which served as metabolic substrates. For further details of technique reference should be made to Boswell and Whiting (1938) and Boswell (1945).

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With the following exceptions the chemical substances used were purchased from commercial sources: perbenzoic acid was prepared from benzoyl peroxide by the method of Gèza Braun (1933), hydrogen peroxide-urea by the method of Chia-Si Lu, Hughes, and Giguère (1941), oxaloacetic and dihydroxymaleic acids, Boswell (1945), and the α-ketoglutaric acid was a gift from Professor R. D. Haworth.

The rates of respiration were calculated as μl./g. dry weight/unit time. In certain experiments it was observed that the dry weight of the tissue at the end of the experiment was abnormally low. The cells appear to contain a considerable amount of water-soluble material which diffuses out into the surrounding medium, following an increase in cell permeability as the result of the presence of certain respiratory inhibitors or the complete destruction of the mechanism of permeability control by dilute H_2SO_4. The substances which inhibit respiratory activity can be divided into two groups. The first group contains malachite green and sodium monoiodoacetate which increase cell permeability. The second group contains potassium cyanide, sodium fluoride, and sodium azide which are without effect upon the permeability in concentrations which markedly reduce the rate of respiration. It would therefore appear reasonable to suggest that the control of cell permeability is located in a particular part of the respiratory mechanism and only when that particular system is inhibited does increased cell permeability develop with loss of cell contents. Malachite green and iodoacetate are dehydrogenase inhibitors and it would appear therefore that the permeability control mechanism is associated with dehydrogenase systems. It is of interest to note certain observations which have been recorded by other workers on the relationship between the accumulation and retention of dissolved substances within the cell and the action of dehydrogenases. Stiles and Jørgensen (1917) showed that potato slices lose electrolyte rapidly in the presence of certain substances including urethane, a known dehydrogenase inhibitor. From the report of Commoner, Fogel, and Muller (1943) it would appear that the four-carbon dicarboxylic-acid system, which is controlled by dehydrogenases, is linked with salt absorption. Lastly, there is the relationship between respiration and salt absorption demonstrated by Machlis (1944) using respiratory inhibitors such as iodoacetate.

In view of the effect of substances such as malachite green upon the dry weight of the tissue slices in those experiments in which they are used, a factor was calculated so that the respiratory activity could be determined in terms of the probable dry weight of the slices at the beginning of the experiment.

I. ASCORBIC ACID OXIDATION

Amounts of ascorbic acid up to approximately 2 mg. were added to about 1 g. fresh weight of washed slices in phosphate buffer solution and the effect of the addition on the rates of oxygen uptake and carbon dioxide output recorded. Fig. 1 illustrates the effect of adding 2·1 mg. and shows that even after 3 hours the stimulation of the respiration rate is maintained about 30 per cent. above the pre-addition value. The total excess oxygen uptake is 186 μl., equivalent to almost 1·4 atom oxygen per mol of added ascorbic acid. The terms 'excess oxygen uptake', 'excess carbon dioxide output', and 'excess R.Q.' are defined as the amount of each gas above the pre-addition level and the ratio between the two.

Even 4½ hours after the addition of ascorbic acid the rate of oxygen uptake remains above the pre-addition level, and in fact no experiment has been continued to the point, if indeed such exists, at which the rate of oxygen uptake falls to the initial value. It is not possible therefore to calculate the excess oxygen uptake exactly in terms of the amount of ascorbic acid added, but it can be stated that for experiments of equal duration the larger the amount of ascorbic acid added the greater the excess oxygen uptake (Table I). There is some variation from day to day in the ascorbic acid oxidizing power of the tissue per unit dry weight which is in part related to the period during which the tissue has been washed prior to use, in part to the total respiratory activity of the tissue, and to other, as yet unrecognized, causes.

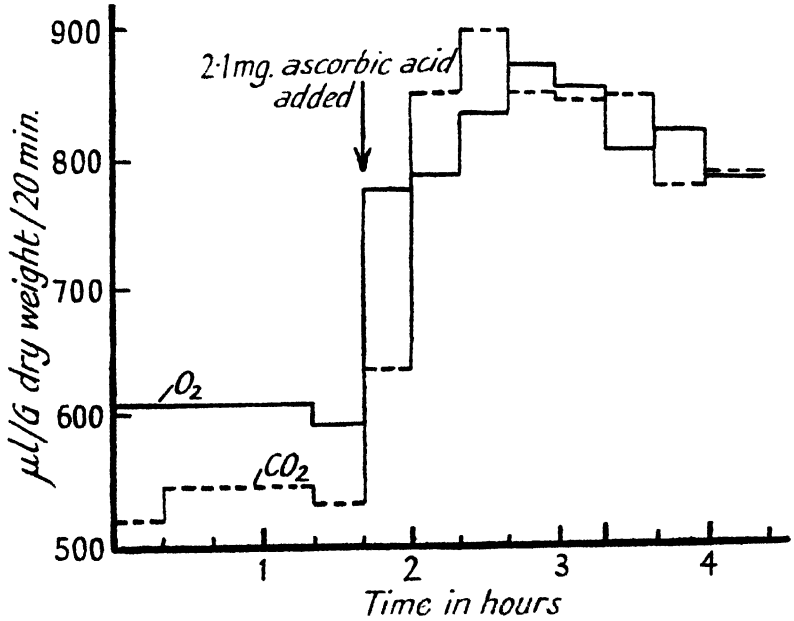


FIG. 1. Effect of ascorbic acid on respiration.

Not only is the rate of oxygen uptake increased by the addition of ascorbic acid but there is also an increase in the rate of carbon dioxide output; this increase is maintained at a value in excess of the pre-addition rate throughout the longest experiment, and the larger the amount of ascorbic acid added the larger is the excess carbon dioxide output. This increase is not, however, proportional to the increased oxygen uptake, and the excess R.Q. falls with increased additions of the acid.

TABLE I

Increased Respiratory Activity (in Excess of Pre-addition Value) following the Addition of Ascorbic Acid

			Excess	
Expt. No.	Amount of ascorbic acid added (mg.).	Duration of expt. (hrs.).	O_2 uptake $\mu l.$	CO_2 output $\mu l.$
	Slices washed for 2 days.			
49.93	0·74	3	30	25
	1·85	3	89	58
	2·00 + 2·00 mg. oxidized ascorbic acid	3	100	121
47.212	1·05	4	59	69
	2·10	4	107	77
	Slices washed for 5 days.			
46.612	0·84	4	82	54
	2·10	4	142	84

Experiments were conducted in which the ascorbic acid was partially oxidized before being added to the tissue. It is clear from Table I that in the presence of dehydroascorbic acid there is a substantial increase in the excess carbon dioxide output and only a small increase in the excess oxygen uptake.

These observations suggest that the oxidation of ascorbic acid by the tissue is linked to the stimulation of carbon dioxide production through a reaction between the dehydroascorbic acid produced and some substance within the tissue. Further, as a result of this reaction, it may be that ascorbic acid is produced and is available for re-oxidation.

Effects of malachite green and sodium azide

The mechanism of the oxidation of ascorbic acid and the relationship between ascorbic acid and the reactant within the tissue was further investigated using two respiratory inhibitors, malachite green and sodium azide.

Malachite green was added to give a final concentration of 1/4000 in the flask. This completely inhibited the respiration of the tissue after 3 hours. The values recorded in Table II show the effect on the respiration rate of the addition of malachite green and ascorbic acid separately and together.

Under the experimental conditions with ascorbic acid present in excess, the fact that malachite green reduced the rate of oxidation of the acid by one-third suggests that the ascorbic acid was being oxidized along two separate channels, one malachite-green sensitive and the other malachite-green insensitive, either by a direct oxidase or by a metallic, non-biological system. The complete inhibition of the oxidation by sodium azide, Table III, shows that the oxidation is wholly enzymic and that the oxidases concerned were azide sensitive.

TABLE II

Effect of Malachite Green and Ascorbic Acid on Respiration (in Excess of Pre-addition Value)

	O_2				CO_2			
		μl. excess.				μl. excess.		
Time in hours.	% inhibition of O_2 uptake. Malachite green alone.	Ascorbic acid alone.	Malachite green and ascorbic acid.	% inhibition	% inhibition of CO_2 output. Malachite green alone.	Ascorbic acid alone.	Malachite green and ascorbic acid.	% inhibition.
1st	49	68	45	34	11	64	7	89
2nd	75	50	31	38	83	62	18	71
Total	—	118	76	36	—	126	25	80

TABLE III

Effect of Sodium Azide and Ascorbic Acid on Respiration (in Excess of Pre-addition Value)

	O_2				CO_2			
		μl. excess.				μl. excess.		
Time in hours.	% inhibition of O_2 uptake. Na azide alone.	Ascorbic acid alone.	Na azide and ascorbic acid.	% inhibition.	% inhibition of CO_2 output. Na azide alone.	Ascorbic acid alone.	Na azide and ascorbic acid.	% inhibition.
1st	64	40	38	5	33	23	24	+4 (Not significant)
2nd	79	34	10	70·6	62	27	2	92·6
3rd	87	25	0	100	74	31	0	100
Total	—	99	48	52	—	81	26	68

The malachite green added to the tissue slices had only a slight inhibitory effect on their carbon dioxide output during the first hour; in fact, in certain experiments the initial effect is slightly stimulating, the inhibition only appearing at a later stage. During the first hour the effect of the malachite green is probably complex, a combination of the inhibition of the dehydrogenase systems—Quastel and Wheatley (1931)—masked by a continued and uninhibited decarboxylation of the reserve of α-keto acids with the additional changes which result from the disturbance of the permeability relations of the cells. The data in Table II show, however, that the excess carbon dioxide which follows the addition of the ascorbic acid to the tissue is greatly reduced in the presence of malachite green. This suggests that the evolution of the carbon dioxide is either directly linked with or follows upon a dehydrogenase reaction. It may be that the excess carbon dioxide is the product of that part of the oxidation system which is malachite-green sensitive; alternatively, the excess carbon dioxide may result from a reaction between the dehydroascorbic acid and some H-donator in the tissue. This latter possibility would agree with a suggestion made earlier as the result of the first group of experiments. The problem of the sources of the excess carbon dioxide will be considered later in the light of other results.

Sodium azide was added to the slices of tissue to give a final concentration of M/400 and in Table III are set out the data showing the azide action calculated in the same way as those for malachite green recorded in Table II. The form of the oxygen uptake-time relation for the tissue slices in the presence of sodium azide suggests that the oxygen uptake is the result of the action of two oxidases or groups of oxidases. During the first 20 minutes following the addition of the azide the oxygen uptake amounts to only 50 per cent. of the pre-addition value, while during the second 20 minutes the residual oxygen uptake has fallen to 30 per cent. Thereafter the rate drifts slowly downwards, but in no experiment was the whole of the oxygen uptake completely inhibited, a basic rate of about 10 per cent. remained even after several hours. The second phase of the azide inhibition may show the existence of a relatively insensitive oxidase or this oxidase be wholly resistant to azide, the manifestation of its activity decreasing owing to the disorganization of associated systems following the complete inhibition of the azide-sensitive oxidase. The azide inhibition of the carbon dioxide output is such that the R.Q. approximates to 2 throughout the experiment.

During the first hour following the addition of the ascorbic acid to the tissue the excess oxygen uptake and excess carbon dioxide output are only slightly modified by the presence of sodium azide. From the second hour onwards the inhibition develops rapidly and is finally complete. The very small degree of inhibition of ascorbic acid oxidation during the first hour may be an example of the protective action of the excess of substrate on the activity of the enzyme in the presence of an inhibitor, the protection being substantial until a considerable concentration of azide has developed within the cells and there has been a considerable reduction in the amount of ascorbic acid present.

During rapid development of inhibition the ascorbic acid-stimulated excess oxygen uptake and excess carbon dioxide output are affected to the same degree. Thus it would appear probable that carbon dioxide production is linked directly with the azide-sensitive oxidase systems rather than with that part of the oxidation of ascorbic acid which occurs through the agency of a dehydrogenase. The latter would be only indirectly affected by azide and so might continue to operate for some time after the direct oxidase system was partially inhibited. This points to the production of carbon dioxide being due to a reaction between an H-donator in the tissue and the dehydro-ascorbic acid formed by the action of a direct oxidase.

While evidence has thus accumulated of the capacity of the tissue to oxidize ascorbic acid, of the systems by which this is achieved, and of the mechanisms involving carbon dioxide output, it has not been possible to determine what, if any, part ascorbic acid and its oxidase play in tissue respiration.

Sodium diethyl-dithio-carbamate is a highly specific reagent for traces of copper, and since ascorbic acid oxidase is a copper-protein complex its activity might well be inhibited by the carbamate. However, the latter decomposes rapidly in the presence of a phosphate buffer at pH 5·5 with the evolution of some gas. Hence, while some inhibition of oxygen uptake by the tissue appears to take place in the presence of carbamate, the gaseous changes which result from carbamate decomposition make it impossible to secure results from which conclusions of any value can be drawn.

Possible hydrogen donators

If ascorbic acid and its oxidase form part of a cyclic system whereby oxygen is absorbed and carbon dioxide given out, it would be of interest to examine the nature of the H-donator by which the dehydro-ascorbic acid is reduced and the cyclic action of the system maintained. In view of the results obtained by James and his co-workers (1944) on the ascorbic acid-oxidase system in barley leaves, certain experiments were carried out using lactic and malic acids and hexose diphosphate. The results are set out in Table IV.

Table IV

Effect of certain H-donators on Ascorbic Acid Oxidation (Values in Excess of Pre-addition Value)

Expt. No.	Ascorbic acid alone.		Additional H-donator.		Ascorbic acid after additional H-donator.	
	O_2	CO_2	O_2	CO_2	O_2	CO_2
			μl. excess.			
47.412						
Lactic acid .	185	214	24	43	113	56
Malic acid .	—	—	38	79	133	95
48.411						
Hexose diphosphate .	162	64	0	0	155	19

In this series of experiments the H-donator to be tested was added to the tissue followed 1 hour later by 2·0 mg. of ascorbic acid. In all cases the excess oxygen uptake following the addition of ascorbic acid was less in the presence of the possible H-donators than in their absence. With malic and lactic acids which stimulate the respiratory activity of the tissue the total excess oxygen uptake for both H-donator and ascorbic acid was less than that for the ascorbic acid alone. The excess carbon dioxide output values were reduced to an even greater extent than those of the excess oxygen uptake, which suggests that the added substances actually reduced the supply of naturally occurring H-donator, thereby slowing down the rate at which the cyclic oxidation and reduction of the ascorbic acid occurred.

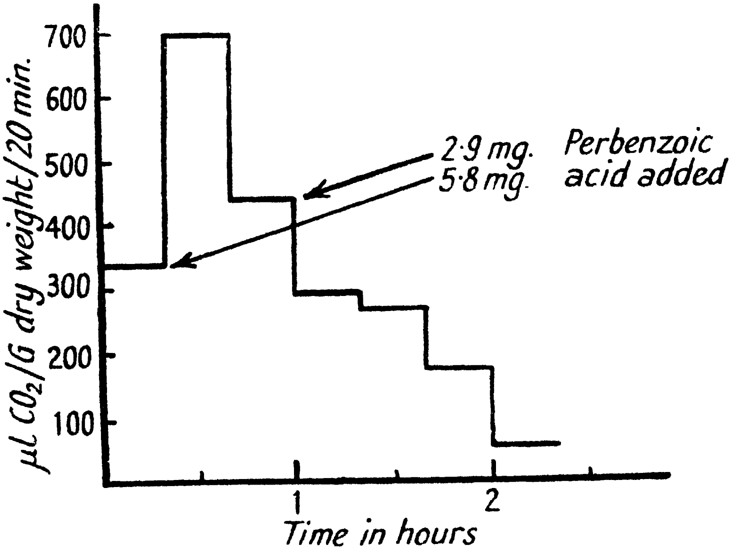


FIG. 2. Effect of perbenzoic acid on carbon dioxide output under anaerobic conditions.

It is not proposed to consider these observations further in this paper since it is clear that the gaseous exchanges are the aggregate of a complex group of reactions. An extensive biochemical investigation has been started to secure data from which further analysis of the mechanisms may be possible.

II. CATALASE AND PEROXIDASE SYSTEMS

As expected from all previously reported observations, when small amounts of hydrogen peroxide were added to tissue slices under anaerobic conditions the peroxide was decomposed by catalase. This also occurred when the hydrogen peroxide-urea complex was used. Clearly while this complex forms stable crystals it is strongly dissociated in aqueous solution. The possibility was considered of using compounds which might be decomposed slowly in the presence of the tissue with the liberation of what might be considered to be nascent hydrogen peroxide. Inorganic peroxides and percarbonates are unstable within the pH limits imposed by the presence of living tissues. Further, ammonium persulphate had no significant stimulating effect upon the rate of carbon dioxide output when added to the tissue under anaerobic conditions. It was therefore necessary to consider the use of organic compounds. Perbenzoic acid was prepared and added to the tissue under anaerobic conditions. Fig. 2 shows that there was an initial stimulation of the carbon dioxide output, that this was followed quickly by a steep fall, and that this fall was not arrested by a second addition of perbenzoic acid. The low level of the carbon dioxide output at the end of 2 hours suggested that the perbenzoic acid had had a lethal effect upon the tissue. In the absence of this substance slices of swede root endure anaerobic conditions for several hours without any marked fall in the carbon dioxide output. Moreover, the bleached appearance, the flaccid state of the slices, and their low dry weight/volume ratios all support the view that the perbenzoic acid is toxic to the tissue.

TABLE V

The Effect of Sodium Dihydroxymaleate alone and in combination with certain Respiratory Inhibitors

Amount added (mg.).	Inhibitor.	Excess gas (μl.).	
		O_2	CO_2
2·05	None	100	160 +88 bound
1·00	,,	52	67+13 ,,
1·23	,,	48	75 total
1·23	Malachite green 1/4000	21	63 ,,
1·55	None	65	120 ,,
1·55	Sodium azide M/400	60	124 ,,

In view of the statement that peroxidase and dihydroxymaleic acid-oxidase are identical systems it is proposed at this point to report certain observations on the effects of this acid on swede root. The addition of disodium dihydroxymaleate stimulates both the oxygen uptake and carbon dioxide output (Fig. 3). The stimulation of gaseous exchange is only of short duration and after about 2 hours the rates have returned to the pre-addition levels. This does not suggest that the acid takes part in any cyclic redox system. The values recorded in Table V show that the excess oxygen uptake does not in any case reach the theoretical value of ½ mol oxygen for each mol of acid added, still less the larger values which might be expected with a substance taking part in a cyclic system. Determinations during the course of the metabolism of the acid show that there is a substantial increase in the amount of carbon dioxide bound in a form which can be decomposed with dilute acid; from which it may be deduced that during the experiment there is an increase in the basicity of the contents of the flask; this would occur if the salt were decomposed by decarboxyation with the liberation of sodium ions. The sodium salt of dihydroxymaleic acid appears to be oxidatively decarboxylated by a system which is partially inhibited by malachite green but which is wholly insensitive to sodium azide. There is no evidence that the dihydroxymaleate forms part of a cyclic redox system, and since the oxidation is azide-insensitive it cannot involve peroxidase or any other enzyme with a heavy metal prosthetic group.

Effects of cyanide

Following upon consideration of these oxidase systems it is of interest to note the effect of cyanide upon the respiration rate of slices of swede root (Table VI). Some observations made using potato tuber and carrot root slices are included for purposes of comparison (Table VII). In those flasks which contained sodium hydroxide in the centre cup the calculated amount of cyanide was mixed with the soda solution.

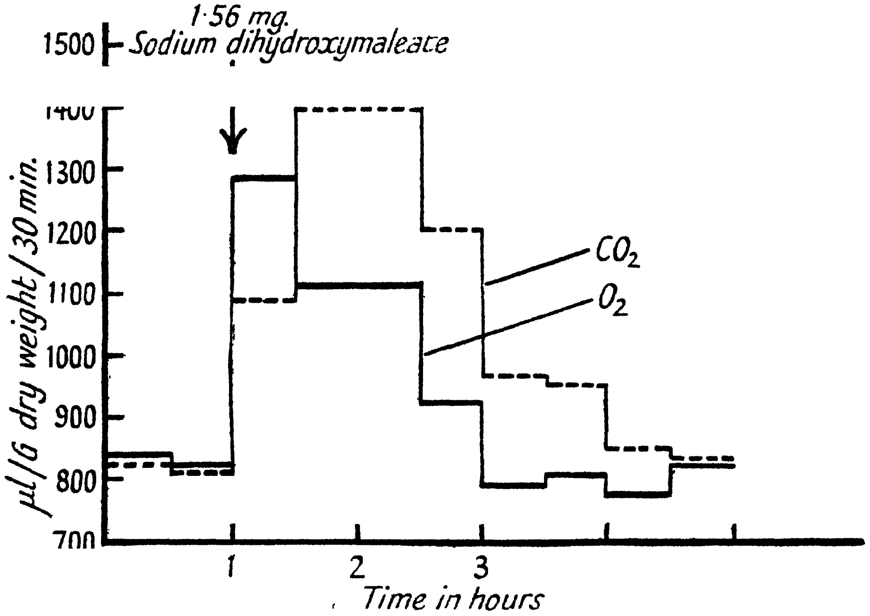


FIG. 3. Effect of sodium dihydroxymaleate on respiration.

A double effect of cyanide upon respiration of swede slices can be noted, namely, that low concentrations stimulate oxygen uptake and carbon dioxide output while higher concentrations have an inhibitory effect upon both. The increase in the percentage inhibition of carbon dioxide output when M/240 KCN is used in comparison with the inhibition at M/48 is as yet unexplained, but is a regular and well-defined feature of these experiments.

The observations in Table VII were made using maincrop potatoes; with early varieties the general effects are the same but concentrations which just depress oxygen uptake and carbon dioxide output in the main crop while depressing the oxygen uptake actually stimulate the carbon dioxide output of the early varieties. While the results set forth in Tables VI and VII cannot be closely compared, as they are not for identical time intervals or concentra-

TABLE VI

Effect of KCN *on the Respiration of Swede Root Slices*

Final concentration of KCN.	Duration of experiment (hrs.).	Respiration rate during final hour as percentage of 'tissue only' value.	
		O_2	CO_2
M/48	4	33·0	55·0
M/240	4	42·7	49·2
M/800	4·5	119·5	126·0
M/2000	4·5	113·5	109·5

TABLE VII

Effect of KCN *on the Respiration Rate of Slices of Potato Tuber and Carrot Root*

Tissue.	Final concentration of KCN.	Respiration rate during 2nd hour after addition as percentage of the 'tissue only' value.	
		O_2	CO_2
Potato . .	M/500	45·5	85·8
	M/1000	78·8	124·0
	M/5000	93·8	129·0
Carrot . .	M/400	32·2	89·2
	M/1000	35·0	130·0

tions, nevertheless certain general conclusions can be drawn. With concentrations greater than M/500 KCN there is always a strong inhibition of oxygen uptake, indicating that part at least of the direct oxidase systems concerned contain a heavy metal prosthetic group. This is in agreement with the existence in the potato and carrot of the polyphenol-oxidase system and with the observation that in the slices of the former at least two-thirds of the respiratory activity is controlled by that system. While the oxygen uptake of the swede tissue is partially inhibited by such concentrations of cyanide, there are nevertheless very clearly marked differences between the reaction of this tissue and those of carrot and potato. First, the rate of oxygen uptake by the swede is much less sensitive to cyanide than that of the other tissues, and second, the rate of oxygen uptake by the swede is actually stimulated by concentrations of cyanide of less than M/800 while with potato M/5000 has a distinctly inhibiting effect. This suggests that the swede contains a cyanide-insensitive direct oxidase system which may or may not be directly stimulated by cyanide but which plays a major part in the oxygen uptake mechanism of the normal slices. It cannot be assumed that such a system is absent from the potato and carrot, but if present it is masked by the dominance of the cyanide-sensitive system.

The effect of cyanide upon the carbon dioxide output from these three tissues is much more uniform; concentrations greater than M/500 are inhibitory, but lower concentrations stimulate the output strongly. In the case of the potato and carrot cyanide possibly acts upon the rate of production of the intermediates which on decomposition give carbon dioxide. It is improbable that the cyanide effect on the potato slices can be due to its action upon the starch-sugar equilibrium, since there is no evidence to support the view that the limiting factor in the respiration of sliced tissue is the sugar content. Hanes and Barker (1931) using whole potatoes found that the stimulation of the rate of respiration by very dilute HCN in the air-stream was correlated with the increased sugar content of the tubers. It was suggested from their observations upon the effect of cyanide in stimulating the activity of amylase *in vitro* that this was the key to the stimulating effect of cyanide upon the respiration rate of the tubers. Other observations have already made it clear that while the actual respiration mechanisms in the whole tubers and in the slices are probably alike, the factors limiting the rates in the two tissues are quite different. For example, the dominant position of protein metabolism in the respiratory activity of slices of potato tuber has already been demonstrated (Boswell, 1945). With swedes there exists a close relationship between the stimulating effect of cyanide upon the rate of oxygen uptake and of carbon dioxide output and it is not possible to determine the identity of the limiting reaction which is stimulated by the addition of cyanide. It is clear also that in the swede at cyanide concentrations of about M/800 any inhibitory effects are completely masked by the strong stimulation of both oxygen uptake and carbon dioxide output.

III. OTHER METABOLITES

In other experiments a wide range of substances was used in order to obtain information concerning their activity as possible intermediate metabolites. Because of the technique of suspending the tissue slices in a potassium phosphate solution and of allowing a time interval of at least 1 hour before the first readings of the manometers were taken, the salt effect which has been studied with carrot slices, if it occurs with swede tissue, is not recorded. Whiting (1938) found no salt effect with potato tissue. The results which are set forth in the following tables show therefore only the effect of the organic ions of the compounds used.

Organic acids of the tricarboxylic acid cycle

The first group contained the four-carbon dicarboxylic acids and certain substances associated with them in what may be described as the 'citric acid cycle' without prejudging the issue as to whether or not the cycle actually exists in the tissue. The acids were added to tissue suspended in buffers at two separate pH values; the results are recorded in Table VIII.

It is clear that the tissue is capable of utilizing these acids in some part of its metabolic system and that with the exception of citric acid the substances are metabolized more actively at pH 4·5 than at 5·5. This may be due either to a connexion between pH and permeability or to the action of pH in determining the degree of ionization of the organic acids added to the external solution, with the consequent regulation of the concentration of the effective ion. Of course, a combination of both effects may occur.

TABLE VIII

Effect of Certain Organic Acids upon the Rate of Respiration of Slices of Swede Root (Values in Excess of Pre-addition Rate)

Acid added . . .	Citric.	α-keto-glutaric.	Succinic.	Malic.	Oxaloacetic.	Pyruvic.
			Buffer pH 5·5			
Concentration in flask as $M \times 10^{-3}$/l. . . .	4·85	3·04	4·24	4·48	3·05	5·25
Excess gas in μl. during 2 hrs.						
O_2	58	4	13	34	11	9
CO_2	169	29	24	76	49	27
Excess R.Q. . . .	2·9	7·2	1·8	2·2	4·5	3·0
			Buffer pH 4·5			
Concentration in flask as $M \times 10^{-3}$/l. . . .	2·29	3·04	3·20	2·98	3·65	6·03
Excess μl. during 2 hrs.						
O_2	102	121	61	106	24	32
CO_2	172	218	107	186	153	127
Excess R.Q. . . .	1·68	1·80	1·75	1·75	6·4	4·0

The high excess R.Q. values for pyruvic and oxaloacetic acids at both pH levels indicates the activity of enzymes which decarboxylate these acids if indeed the decarboxylation of oxaloacetic acid is an enzymic reaction. The decomposition of α-ketoglutaric acid is recorded in the literature as an oxidative decarboxylation with an R.Q. of 2. From Table VIII it may be seen that a very wide gap exists between the excess R.Q. at pH 5·5 and that at 4·5 when this acid is used as a metabolite. This may be explained if consideration is given to the fact that at the higher pH of 5·5 the rate of metabolism is low, the gaseous exchanges being very small in comparison with the values at pH 4·5. If in the dehydrogenation of the acid the hydrogen is retained even in part by the initial hydrogen acceptor and not passed immediately and completely to a terminal direct oxidase system, then the recorded oxygen uptake will not measure the oxidation of the acid and the excess R.Q. will be too high. Now at pH 5·5 the excess gaseous exchange values are so small that the retention of only a little hydrogen by an intermediate acceptor would seriously distort the excess R.Q. value, while at 4·5 a similar retention would have very little effect upon the excess R.Q. value. For example, an increase in the recorded oxygen uptake of 12 μl. following the addition of α-ketoglutaric acid at pH 5·5 would reduce the excess R.Q. from 7·2 to 1·8, while at pH 4·5 the excess R.Q. would be reduced only from 1·8 to 1·64. This point is of general application, namely, that the excess oxygen uptake is not necessarily a complete measure of the oxidation of the added substrate and also that where the total gaseous exchanges are small the magnitude of the excess R.Q. value has no very great significance. No importance should therefore be attached to the differences between the excess R.Q. values recorded for oxaloacetic and pyruvic acids at pH 4·5 and 5·5 since in both cases the excess oxygen uptake values at pH 5·5 are too small to have been an accurate measure of the oxidation of these substrates. The only result which cannot be explained on this basis is with citric acid, where the values at both pH are large enough to be significant and yet a very considerable difference exists between the excess R.Q. values.

With citric, α-ketoglutaric, succinic, and malic acids at pH 4·5 the excess gaseous exchanges are sufficiently large to make the values significant and the uniformity of the excess R.Q. values is most striking. When consideration is given to the excess R.Q. values which result from the complete oxidation of these acids it is clear that the reactions into which they enter involve predominantly decarboxylation with the formation of organic residues which are not completely oxidized. It cannot be ruled out that these acids act as catalysts, but there is no evidence of the existence of the citric acid cycle. This can only be further examined when data are available of the chemical reactions into which these substances enter. The results obtained using fumaric acid are almost identical with those for malic acid and leave no doubt as to the existence of an active fumarase.

The prior addition to the tissue of malachite green to give a final concentration of 1/1500 completely inhibited the stimulation of respiratory activity which follows the addition of citric, α-ketoglutaric, succinic, fumaric, and malic acids to the tissue in the absence of the dyestuff. This shows that the excess oxygen uptake which follows the addition of these acids to normal slices is associated with dehydrogenases. It appears that the decarboxylation which yields the carbon dioxide is either preceded by or coincident with dehydrogenation. With oxaloacetic and pyruvic acids the prior addition of the inhibitor prevents any stimulation of the oxygen uptake on the subsequent addition of the acids, but the excess carbon dioxide output is only very little less than the value secured in the absence of the inhibitor. This suggests that whatever part these acids may have in a metabolic system, decarboxylation can occur independently of dehydrogenation and also that the excess oxygen uptake which follows their addition to the normal tissue is associated with a dehydrogenase system. This does not eliminate the possibility that in normal tissue the metabolism of pyruvic acid is an oxidative decarboxylation.

Amino-acids

The second group of substances examined consists of the amino-acids, glutamic and aspartic acids, alanine, glycine, and tyrosine, and the amide asparagine. Observations made using these substances are recorded in Table IX. It may be noted that Boswell (1945) has recorded that this group of substances had no effect upon the rate of respiration of washed slices of potato tuber.

These values were obtained using a buffer at pH 4·5; at 5·5 the excess oxygen uptake and carbon dioxide output values with glutamic and aspartic acids and asparagine were all very much lower and the excess R.Q. values larger, while

TABLE IX

The Effect of Certain N *Compounds upon the Rate of Respiration of Slices of Swede Root (Values in Excess of Pre-addition Rate)*

Acid added	Glutamic	Aspartic	Alanine	Glycine	Tyrosine	Asparagine
Concentration in flask as $M \times 10^{-3}$/l.	4·15	4·17	5·75	6·8	1·50	4·29
Excess μl. during 2 hrs.						
O_2	106	80	12	10	−14	41
CO_2	180	142	13	7	−17	71
Excess R.Q.	1·71	1·78	1·08	0·70	—	1·74

those with alanine and glycine were little affected. It is to be noted that while glutamic and aspartic acids and asparagine stimulate the rate of respiration to a very considerable extent, alanine and glycine have very little effect and tyrosine is slightly inhibitory, the rate of respiration following addition falling below the 'tissue only' level. The difference between aspartic acid and asparagine as stimulators of respiratory activity is probably due to the permeability relations of the two substances. To examine the possibility that the metabolism of glutamic and aspartic acids is through an amino-acid oxidase, these amino-acids were added to tissue whose respiratory activity had been partially inhibited by the prior addition of malachite green. The results are set out in Table X. It is clear that the oxidations of glutamic and aspartic acids are achieved by a dehydrogenase system and not by a direct oxidase. It is of interest that glutamic acid is capable of partially removing the inhibition of carbon dioxide output resulting from the action of malachite green, an action comparable to that of pyruvic acid. The non-oxidative decarboxylation of pyruvic acid is a well-known reaction and it would appear therefore that under these experimental conditions glutamic acid is being decarboxylated in a non-oxidative reaction. This is in accord with the results of Schales and Schales (1946), who have prepared a glutamic acid decarboxylase from a wide range of plants including turnip and swede.

TABLE X

The Action of Certain Substances upon the Degree of Inhibition of Respiratory Activity caused by Malachite Green in a Final Concentration of 1/15,000

	No organic compound.		Glutamic acid.		Aspartic acid.		Pyruvic acid.	
	O_2	CO_2	O_2	CO_2	O_2	CO_2	O_2	CO_2
Tissue only	1·00	1·00	1·00	1·00	1·00	1·00	1·00	1·00
Malachite green added	0·54	0·85	0·54	0·85	0·55	0·86	0·55	0·92
Organic compound added	0·28	0·42	0·32	0·53	0·33	0·53	0·33	0·51
	0·17	0·28	0·13	0·64	0·13	0·24	0·09	0·89
	0·13	0·17	0·13	0·30	0·11	0·10	0·10	0·70

The respiration rates are shown as ratios of the initial 'tissue only' values and pyruvic acid is included for the purpose of comparison.

Other organic acids and acetaldehyde

The third group of substances includes lactic, tartaric, oxalic, and formic acids and acetaldehyde. Of the four acids formic has an inhibitory action on both oxygen uptake and carbon dioxide output, the treated tissue becomes flaccid, and the dry weight per volume ratio is low. Table XI shows the stimulating effect on respiration of lactic, tartaric, and oxalic acids with particular reference to the influence of the pH at which they are applied. The nature of the metabolic processes in which these acids are involved remains to be investigated later, but certain points may be noted. The high excess R.Q. with oxalic acid suggests complete oxidation to carbon dioxide and water; little significance can be attached to the value at pH 6, since with an excess oxygen uptake of only 8 μl. the accurate determination of oxidation is impossible. The use of malachite green in the usual way shows that in all cases the oxidation is through a dehydrogenase system and the uptake of oxygen and the output of carbon dioxide are the products of closely linked reactions.

TABLE XI

The Effect of Certain Organic Acids on the Rate of Respiration of Slices of Swede Root (Values in Excess of Pre-addition Rate)

pH.	Acid added.	Amount (mg.).	Excess in μl.		Excess R.Q.	Duration of expt. (hrs.).
			O_2 uptake.	CO_2 output.		
4·5	Oxalic	2·1	33	120	3·6	2
6·0		2·0	8	48	6·0	4
4·5	Tartaric	2·5	70	156	2·2	4
6·0		2·0	10	30	3·0	4
4·5	Lactic	2·0	39	90	2·3	3
6·0		2·1	13	30	2·3	3

Acetaldehyde depresses irreversibly the rate of oxygen uptake immediately after addition. The rate of carbon dioxide output is strongly stimulated in the early stages, the R.Q. rising to about 2. Later, carbon dioxide output is reduced, and after 3 hours both oxygen uptake and carbon dioxide output are below the 'tissue only' value and the R.Q. has fallen to 1·25. When acetaldehyde is added to the tissue after malachite green the inhibition of the oxygen uptake is much more rapid than with the malachite green alone, there is not a proportionate reduction in the output of carbon dioxide and the R.Q. rises steadily. It is of interest to note that as in the case of the potato tuber tissue acetic acid has a depressor action on the rate of respiration and a similar effect with barley roots has been observed.

Effect of iodoacetic acid

Iodoacetic acid has been shown to inhibit several dehydrogenases by acting on the —SH group. When added to tissue slices in a final concentration of $1\cdot25\times10^{-2}$M, inhibition of respiratory activity is complete after 1½ hours, and at $1\cdot25\times10^{-3}$M the oxygen uptake is reduced to 10 per cent. and the carbon dioxide output to 25 per cent. of the pre-addition values after 3 hours, all at pH 5·5. The degree of inhibition is related to the pH value, decreasing as the acidity decreases. Citric, succinic, pyruvic, aspartic, and glutamic acids were added to tissue treated half an hour earlier with sodium monoiodoacetate in a final concentration of $1{\cdot}25 \times 10^{-3}$M. With citric, succinic, and aspartic acids the inhibition of oxygen uptake and carbon dioxide output was not removed. With pyruvic and glutamic acids only the carbon dioxide output was stimulated to a considerable extent and that only temporarily, in the case of glutamic acid the carbon dioxide output being above the pre-inhibition value for a short time. These observations are in line with those made previously with another dehydrogenase inhibitor, malachite green, from which it was deduced that with citric, succinic, and aspartic acids the production of carbon dioxide is linked with dehydrogenases, while with pyruvic and glutamic acids direct decarboxylation may occur.

Effect of sodium fluoride

Sodium fluoride in a final concentration of $1{\cdot}25 \times 10^{-2}$M was used as a respiratory inhibitor. After 4½ hours the rate of oxygen uptake was 29 per cent. of the pre-addition level and the R.Q. had risen to 1·58. The data set forth in Table XII show that the acids of the 'citric acid' cycle are all capable of reducing the inhibition of respiration produced by the addition of sodium fluoride. Except with succinic and citric acids the rate of respiration is raised above the pre-addition level, but with these two acids only the rate of carbon dioxide output passes this level. That succinic acid should be one of the two less effective acids is of interest in view of Borei's (1945) observation that while sodium fluoride inhibits the enolase in the hexosephosphorylation cycle it also inhibits the cytochrome-oxidase system. Since these acids remove or reduce the fluoride inhibition it would appear that their part in the respiration system follows upon the phosphorylation cycle, the products of glycolysis being the

TABLE XII

Respiration Inhibited by the Addition of Sodium Fluoride to a Final Concentration of $1{\cdot}25 \times 10^{-2}$*M Forty Minutes before the Addition of the Organic Acids*

Acid	Malic.	Pyruvic.	Succinic.	Citric.	Oxaloacetic.
Concentration $10^{-3} \times$ M	4·6	5·8	4·6	4·6	4·5
Excess gaseous exchange in µl. during 2 hours over the respiration rate following the addition of fluoride.					
O_2	112	120	109	81	91
CO_2	103	124	94	96	153
R.Q.	0·92	1·04	0·86	1·18	1·68
Excess gaseous exchange in µl. during 2 hours over the respiration rate before the addition of fluoride.					
O_2	57	66	—	—	10
CO_2	75	97	58	22	65
R.Q.	1·32	1·47	—	—	6·5

substrates for the organic acid metabolic reactions. This would be additional support for the schemes suggested by Gregory and Sen (1937) and Steward and Street (1947).

Comparison between the data in Tables VIII and XII reveals the different metabolic reactions in which the organic acids are involved in the presence and absence of sodium fluoride. In the presence of fluoride, while the rates of metabolism are of the same order as in its absence at pH 4·5, the excess R.Q. values are much smaller even in the case of oxaloacetic acid. This supports the view, based upon the high R.Q. values for fluoride-treated tissue, that fluoride inhibits the production of oxidizable substrates. These can be replaced by certain organic acids and the fact suggests that a considerable proportion of the respiratory activity of the normal swede slices is directed through the metabolism of organic acids.

Discussion

It is proposed in this section to consider the observations which have been made using the swede 'root' (*Brassica napus*) and to compare them with those recorded by Boswell and Whiting (1938) and Boswell (1945) from the potato tuber.

It must be emphasized that any conclusions set forth are only valid with reference to washed, sliced tissue, and that they do not necessarily give a picture of the respiratory mechanism for the whole tuber or root. From the work of Steward it is clear that slicing and washing with adequate aeration alters the balance of the respiratory mechanisms. This is partly due to the increased oxygen supply and partly to the initiation of certain chains of reactions associated with meristematic activity. For example, those which maintain the constant level of protein nitrogen in the whole mature organ become distorted in such a way that the rate of protein synthesis is stimulated without a proportionate increase in the rate of protein catabolism; thus an increased protein content results. Further, it is clear from the work of Berry and Steward (1934) that different storage tissues react to wounding with widely different intensities.

When attempting to draw a comparison between the respiratory mechanism of slices of swede root and of potato tuber certain metabolic differences are clear. The most important of these relates to the nature of the direct-oxidase systems through which hydrogen is transferred to molecular oxygen. In accordance with previous reports it is confirmed that swede root does not contain the polyphenol oxidase system. A complete identification of the direct oxidase systems in swede root has not yet been achieved. The form of the respiration-time curve following the addition of ascorbic acid, excess oxygen uptake values greater than one atom oxygen per mol of ascorbic acid added, the effects of dehydroascorbic acid, of sodium azide, and malachite green, all suggest that ascorbic acid takes part in a cyclic redox system. In this the uptake of oxygen is associated with the activity of a direct oxidase and the liberation of carbon dioxide is connected with the reduction of dehydroascorbic acid. No evidence is available as to the nature of the H-donator involved in this reduction. Under the experimental conditions with an excess of ascorbic acid present there is evidence that part of the added ascorbic acid is oxidized by a dehydrogenase system. This is comparable to that which occurs in the potato tuber, where ascorbic acid appears to be the co-enzyme of a redox system which does not directly involve oxygen.

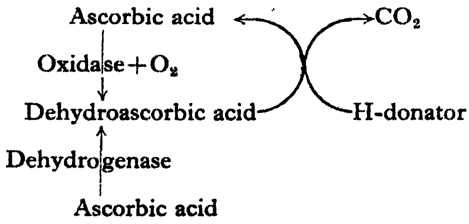
The nature of the other direct oxidase systems in the tissue can as yet only be inferred from their reactions to known specific inhibitors. It is clear that some part of these systems involves one or more enzymes which do not contain a heavy metal in their prosthetic group since they are insensitive to both azide and cyanide. All attempts to determine the position of peroxidase in the respiratory system have failed; it is also certain that as in the potato tuber dihydroxymaleic acid does not form part of a terminal oxidase system.

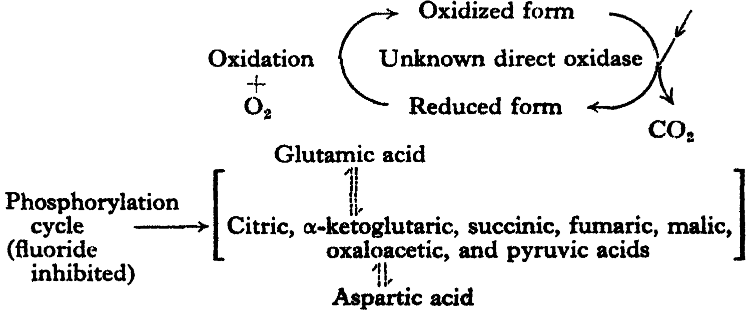
The effect of cyanide in small concentrations in stimulating the rates of both oxygen uptake and carbon dioxide output of swede slices raises the question of the mechanism of the stimulation. In concentrations which do not inhibit enzyme activity, it cannot be ruled out that the cyanide serves as an oxidizable substrate with resulting stimulation of the rate of gaseous exchange. With the potato tuber and carrot root the oxygen uptake mechanism is much more sensitive to cyanide; this is to be expected from the dominant position of the polyphenol oxidase among the terminal oxidases. It may well be that the absence of any stimulation of oxygen uptake in these two tissues is a resultant effect, the marked inhibition of one oxidase system being not wholly compensated for by the stimulation resulting from cyanide operating as a metabolite, which is a possibility in view of the stimulation of the carbon dioxide output.

As with slices of potato tuber the results showed that slices of swede root metabolized the four-carbon dicarboxylic-acids together with pyruvic, citric, and α-ketoglutaric acids. These reactions were apparently controlled by dehydrogenases. The data obtained by adding the organic acids to fluoride-treated tissue indicates that these acids form a metabolic system which uses as an initial substrate the products of the phosphorylation cycle. The respiratory activity of potato tuber slices is only inhibited by concentrations of fluoride which render the tissue somewhat flaccid; this insensitivity is not unexpected since it has been shown that the polyphenol oxidase system is insensitive to fluoride. The evidence available does not permit a decision to be reached as to the existence in the tissue of a system comparable to the tricarboxylic-acid cycle.

In contrast to slices of the potato tuber, the respiratory activity of slices of the swede root is stimulated by the addition of amino-acids and in particular by glutamic and aspartic acids. The metabolism of these two acids, which is very much more vigorous than that of glycine and alanine, may occur along two lines. Glutamic and aspartic acids may take part in transamination systems, and the gaseous exchanges which follow their addition to the tissue may be due to the metabolism of the resulting α-keto acids. The excess R.Q. values for the metabolism of glutamic acid and of the α-ketoglutaric acid which would result from the transamination are so close together that this line of metabolism may occur. With aspartic acid the excess R.Q. values for acid and the corresponding α-keto acid, oxaloacetic, are so far apart that it is unlikely that aspartic acid is metabolized through transamination.

Since it has been shown that these amino-acids are oxidized by the action of dehydrogenases, the alternative line for both amino-acids would be analogous to the scheme set forth previously for the potato tuber. These acids would serve as H-donators to some direct oxidase system, carbon dioxide being evolved in the process and the increased rate of oxygen uptake being due to the increased rate of production of the reduced phase of the oxidase system. The difference between the reactions of potato and swede slices to the addition of these amino-acids would reflect the different factors which limit the rate of respiration in the two tissues. In the potato tuber slices it has been shown that the factor limiting the rate of respiration is the amount of the naturally occurring polyphenol. When this is supplemented by the addition of caffeic acid the supply of amino-acid H-donator becomes the limiting factor and only in that state does the addition of glutamic and aspartic acids stimulate the rate of respiration. If in the original slices the factor limiting the rate of respiration had been the supply of the amino-acid which functions as the H-donator and not of the natural polyphenol, then the addition of the amino-acid would have stimulated the respiratory activity. Glutamic and aspartic acids added to swede tissue have exactly this effect and this is compatible with them acting as H-donators to a direct oxidase system of which the rate of reaction is limited by the supply of reducing reagent. Under such a scheme the difference between the rates of metabolism of glutamic and aspartic acids and of glycine and alanine would be explicable on the basis of the dehydrogenases present in the tissues.




Glutamic or Aspartic acid
Oxidized form
Oxidation + O_2
Unknown direct oxidase
Reduced form
CO_2
Glutamic acid
Phosphorylation cycle (fluoride inhibited)
Citric, α-ketoglutaric, succinic, fumaric, malic, oxaloacetic, and pyruvic acids
Aspartic acid

The observations recorded in this paper are set forth in the following scheme for direct comparison with the scheme of Boswell (1945).

It has been assumed throughout that the conclusions of Boswell that the polyphenol oxidase is the major terminal oxidase in the slices of the potato tuber has found general acceptance and support. However, Schade and his co-workers (1948, 1949) have reached the conclusion from their work on slices and homogenates of the potato tuber that the terminal oxidases in the slices are two, cytochrome oxidase and an unknown oxidase referred to as X which oxidizes *p*-phenylene diamine. They take the view that polyphenol oxidase plays no part in the potato tuber system. The evidence which they present for the presence and function of cytochrome oxidase is completely adequate and there is no doubt that it is one of the direct oxidase systems in the tuber slices. The evidence from which they deduce that the polyphenol oxidase has no part as a terminal oxidase and on which they base their claim to discover enzyme X is defective owing to the absence of critical experiments, and their deductions are quite unsupported by their published results.

In accordance with the observations of all previous workers Schade found that the addition of catechol to the tissue resulted, with a certain exception which can be easily explained, in the stimulation of the oxygen uptake and in all cases this was followed by a rapid fall in the rate of oxygen uptake to some value 30–50 per cent. of the pre-addition value; further, that when a certain quantity of catechol was added the oxidase was completely inhibited and further additions had no effect. Since after overnight washing following the addition of catechol the residual oxygen uptake disappears entirely, Schade dismisses this oxygen uptake as due to the enzymatic oxidation of the catechol by non-viable slice tissue at a rate which diminishes with time. It is probable that had he measured carbon dioxide output as well as oxygen uptake he would have applied some adjective other than 'non-viable' to the tissue. That the residual respiration disappears during the overnight washing is hardly unexpected; in fact, it would have been surprising if the complete inhibition of a substantial part of the respiratory activity with the formation in excess in the tissue of such powerful oxidizing agents as quinones had not disorganized the highly integrated respiratory mechanism with the disappearance of respiration, leaving intact the activity of the oxidases as isolated systems, particularly since it is well known that the dehydrogenases in plant tissue are very sensitive to disorganization. On these results Schade considers that catechol acts as a tissue poison rather than as a specific inhibitor despite his own evidence of the specific inhibition of the oxidase. The distinction between the two effects is difficult to draw since fundamentally any tissue poison must inhibit some part of the respiratory mechanism and it is unimportant whether it does so by acting specifically upon some chemical system or by physical action upon the colloidal complexes. While taking account of the inhibitory action of catechol, Schade makes no reference to the results obtained using non-toxic water-soluble polyphenols such as caffeic acid which stimulate both oxygen uptake and carbon dioxide output without any subsequent inhibition. Nor does he consider the relation of polyphenols to amino-acid metabolism.

Schade attempts to confirm his view that polyphenol oxidase has no part in slice respiration and to demonstrate the presence of the cytochrome oxidase by the use of carbon monoxide. As far as the cytochrome oxidase is concerned the light-reversible inhibition is adequate evidence for its presence, but the conclusions drawn on the non-participation in respiration of the polyphenol oxidase based upon the absence of any light-insensitive carbon monoxide inhibition under the experimental conditions used are wholly without value. For example, detailed results are given to show that in a mixture of 5 per cent. oxygen and 95 per cent. carbon monoxide the inhibition of the respiration rate is wholly reversible by light and cannot therefore be due to polyphenol oxidase. Since other data in the paper show that in 5 per cent. oxygen the rate of respiration is little more than 60 per cent. of the rate in air, all that the results really show is that this residual respiration in 5 per cent. oxgyen does not involve the polyphenol oxidase system. They do not permit any conclusion to be drawn about the nature of the oxidase system involved in that part of the respiration rate which was inhibited by the low oxygen pressure. Observations using a mixture of 20 per cent. oxygen and 80 per cent. carbon monoxide which are clearly of vital importance are very scantily reported. Under these conditions inhibition occurred only occasionally, and when it did so it never exceeded 20 per cent. and was light-reversible. In so far as these results show anything they show the participation of cytochrome oxidase. In view of the irregular occurrence of the inhibition to even a minor degree, it would appear more probable that the failure to demonstrate a light-insensitive inhibition is due to the fact that the concentration of carbon monoxide in the presence of 20 per cent. oxygen is insufficient to inactivate the polyphenol oxidase to the point at which the concentration of the free enzyme limits the rate of respiration, rather than to the non-participation of this enzyme as a terminal oxidase in the respiratory mechanism. Since data in the paper show that polyphenol oxidase is present in homogenates in concentrations such that the oxygen uptake due to its activity is 35 times that due to the cytochrome oxidase, it is to be expected that experimental conditions which only occasionally are sufficient to inhibit the cytochrome oxidase to the point at which the respiration rate is reduced are unlikely to reduce the polyphenol oxidase activity to a similar point.

From the great sensitivity of the rate of tissue respiration to reductions in the partial pressure of oxygen Schade postulated that in addition to the cytochrome oxidase there was present another oxidase which he designated X. This enzyme was regarded as responsible for the oxidation of *p*-phenylene diamine added to the tissue homogenates, since it is known that this substrate is not oxidized by polyphenol oxidase. What was overlooked was the fact that a tissue homogenate was not a pure preparation of the oxidase but a mixture of this enzyme with among other things the naturally occurring polyphenol of the potato tuber. Under the experimental conditions the polyphenol will be oxidized to a quinone which oxidizes *p*-phenylene diamine by a purely chemical reaction. Enzyme X which in the homogenate oxidizes *p*-phenylene diamine is therefore none other than polyphenol-oxidase plus a polyphenol, forming a powerful redox system, the *p*-phenylene diamine acting as an H-donator to the quinone, reducing it to the *o*-dihydroxy state when it is available for re-oxidation by the oxidase. This enzyme X is described as being insensitive to carbon monoxide. When the data are examined it is found that the experiments were carried out using a gas mixture of 5 per cent. oxygen and 95 per cent. carbon monoxide in which the polyphenol oxidase would be relatively inactive, and even so a 20–25 per cent. inhibition occurred which was light-insensitive. Enzyme X is markedly sensitive to cyanide and to azide, which indicates a heavy metal in the enzyme structure.

These results indicate that enzyme X is a crude preparation of polyphenol oxidase and that Schade's observations, far from proving that this oxidase is not one of the terminal oxidases of potato tuber slices, provide additional confirmatory evidence that it is the principal terminal oxidase.

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Studies in the Genus *Elaphoglossum* Schott

I. Stelar Structure in Relation to Habit

BY

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With nine Figures in the Text

ABSTRACT

The habit is described of a number of species of *Elaphoglossum* collected in Jamaica. Species were present with creeping horizontal rhizomes, shortly creeping or ascendent rhizomes, and one species with an ascendent or upright rhizome.

The stelar structure of these rhizomes is described and it is shown that it bears a relation to the habit of the rhizome.

A preliminary examination of the apices has shown that the symmetry of the rhizome is determined in the initiation of the primordial tissues; it is not secondarily induced by the habit of the rhizome.

The occurrence of dormant buds is noted in all the species examined.

ELAPHOGLOSSUM (Schott, 1834) comprises a genus of Acrostichoid ferns, many of which have also been included in the genus *Acrostichum* itself. As a genus it is remarkable for the uniformity of the frond which, with few exceptions, is undivided and ovate-lanceolate or lanceolate in shape, both in the sterile and fertile state. The genus is more or less confined to the temperate mountain regions of the tropics, where some species ascend to 3,500 metres and higher. In addition, there are a few species in outlying islands such as Tristan da Cunha, Amsterdam Island, and the Azores. The general distribution of the genus is shown in Fig. 1, which is compiled from Christ (1900). It is interesting to note here that, although reaching as far south as Patagonia and south Chile, *Elaphoglossum* has nevertheless no representatives in the islands of Juan Fernandez which have an otherwise very rich flora of ferns, including the important endemic *Thyrsopteris elegans*. The northern limit of the genus is reached in the Azores, where a single species, *E. hirtum* (Swartz) C. Ch., grows in vegetable debris on the branches of trees and on rocks in the Juniper forest at an altitude of 1,200 to 1,500 metres (Boatman and Marler, 1949).

The representation of the genus in the Old World is very poor compared with that in the New. The greatest number of species occur in the Andean and sub-Andean regions, from Colombia and Venezuela to Bolivia and Brazil. These Andean species show a wide range of adaptation in relation to their habitat. The species of high altitudes are of lowly stature and usually possess a thick investment of imbricate scales. Those growing on the lower slopes are

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usually larger, with laxer fronds and scales that are often early deciduous, leaving the mature frond almost glabrous.

The most recent comprehensive systematic account of the genus is by Christ (1900), although since that time numerous new species have been described. The small variation in external morphology within the genus renders the taxonomic problem very difficult and the differences between the species, which lie principally in the form of the scales, are both critical and minute. The present state of the systematics of *Elaphoglossum* is far from satisfactory, but it is difficult to see how any substantial advance can be made until there is a clearer knowledge of the phylogenetic trends within the genus. This cannot be achieved by a study of the external morphology alone.

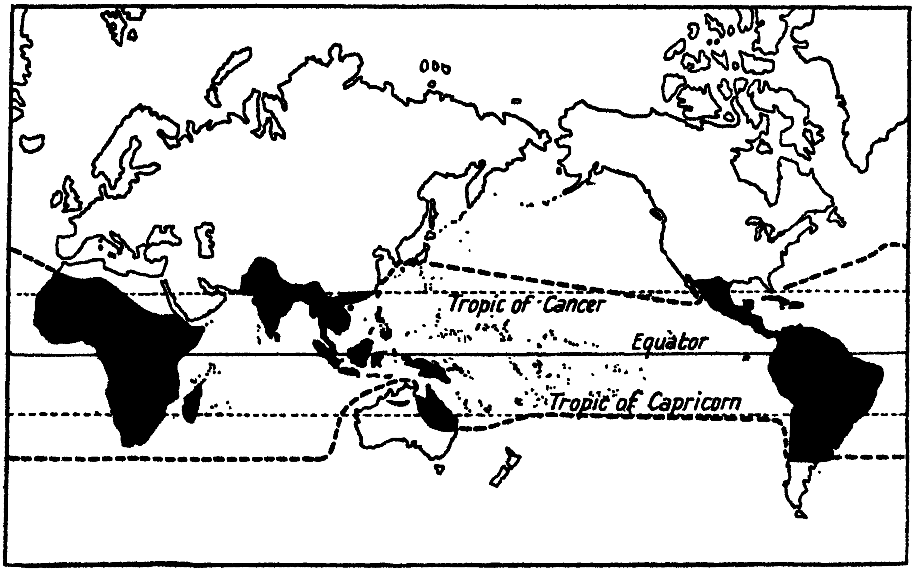

FIG. 1. *Elaphoglossum* Schott. Distribution of the genus.

MATERIAL

The material that has been used in the present investigations was collected in the Blue Mountains of Jamaica in 1939 by H. Hamshaw Thomas and W. R. Philipson. The author is indebted to Dr. Thomas for passing on to him the preserved material and to Dr. Philipson and the British Museum (Natural History) for the loan of herbarium specimens. The preserved material, with the exception of *E. chartaceum*, was named by matching with herbarium specimens made at the time of collection and subsequently determined by W. R. Maxon. As there was no dried specimen of *E. chartaceum* this plant was named from other herbarium material. Nine species were represented in the collection, namely, *E. latifolium* (Swartz) J. Sm., *E. tectum* (H. and B.) Moore, *E. Eggersii* (Baker) Christ, *E. Sellowianum* (Presl) Moore, *E. chartaceum* (Baker)

C. Ch., *E. muscosum* (Swartz) Moore, *E. hirtum* (Swartz) C. Ch., *E. pallidum* (Baker) C. Ch., and *E. villosum* (Swartz) J. Sm.

HABIT OF THE RHIZOME

All the species investigated were collected in the Rain Forest Zone at an altitude of from 1,350 to 1,800 metres. The general habitat was vegetable debris either on banks, rotting logs, or branches of forest trees. In some species, such as *E. latifolium*, the petioles of the fronds appear always to ascend. In others, such as *E. villosum*, the fronds may be pendent or the plant may grow on the underside of a branch and the fronds ascend only at the tips. All species possess a rhizome, varying from 1 cm. to 0·5 cm. or even less in diameter, according to the species.

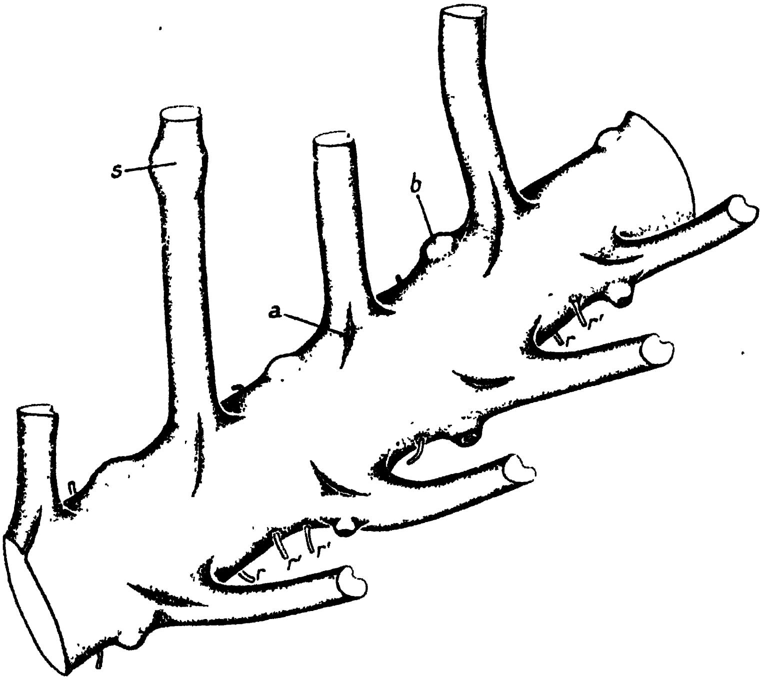

FIG. 2. *Elaphoglossum latifolium.* Portion of the rhizome with the scales removed; *a*, slightly elevated ridge of parenchymatous tissue on the dorsal side of the insertion of the petiole; *b*, bud on the abaxial side of the petiole; *r*, root; *r'*, root in association with bud; *s*, swelling in the petiole, about 2 cms. above the rhizome, at which the old fronds disarticulate.

The habitat of the rhizome of *E. latifolium*, after removal of the close covering of scales from both the rhizome and petiolar bases, is shown in Fig. 2. The rhizome creeps horizontally and bears fronds in two rows, one on each side of the median line with the insertions in each row arranged alternately. The distance between the insertions in one row varies between 1·5 and 2 cm. On the abaxial side of each petiolar base is a conspicuous dormant bud. None of these buds was observed to have undergone any prolonged development, although it is probable that destruction of the main apex would be followed by the development of one or more of these buds. Two or three small roots arranged linearly in an obliquely ventral direction are frequently seen close behind the bud. One of these series is visible in Fig. 2. Other than these small roots associated with buds, the bearing of roots is confined to the ventral surface of the rhizome.

These features of the rhizome of *E. latifolium* are also found in *E. Sellowianum*, *E. tectum*, *E. Eggersii*, and *E. chartaceum*, all of which have horizontal creeping rhizomes. Species differ in the diameter of the rhizome and the distance between the insertions of the petioles, but the general picture remains unaltered.

In *E. muscosum*, *E. hirtum*, and *E. pallidum* the rhizome is not longly creeping, but is shortly horizontal or obliquely ascendent. It is never completely upright. The fronds in these species are not borne in two rows, but are crowded together in several rows on the dorsal side of the rhizome. The insertions tend to form diagonal as well as longitudinal rows, but with no clear regularity. Buds are found only on the abaxial side of those petioles nearest the ventral surface of the rhizome. These buds are much less conspicuous than those found in the previous group of species, but in one large specimen of *E. hirtum*, with a rhizome about 12 cm. long, two of these buds, both about 9 cm. from the apex, had developed into short branches. The parent rhizome was hard and brittle in this region and presumably dead. Its fronds were confined to a distance of about 3 cm. behind the apex; only necrotic leaf bases were present along the rest of the rhizome. The short branches had reached a diameter about half that of the parent rhizome and the fronds upon them about one-quarter of the mature size. With progressive decay of the parent rhizome, these outgrowing buds would no doubt remain as independent plants. As in the former group of species, buds may have two or three rootlets behind them on the parent rhizome, but otherwise roots are again confined to the ventral surface.

In the last species, *E. villosum*, the rhizome is either obliquely ascendent or even completely upright. The fronds are crowded together and inserted all around the rhizome, although there is no evident phyllotaxy. The fronds are also often secund, an effect probably due to the position in which the plant grew. Minute dormant buds are present on the abaxial side of the bases of some of the petioles. These buds are very difficult to detect externally and have never been found to have undergone any development. The roots appear between the petioles and from the bases of the insertions; they are not restricted to any particular part of the rhizome, which is thus devoid of any recognizable dorsal and ventral surfaces.

STELAR STRUCTURE

A reconstruction of the stele of *E. latifolium*, built up from a series of transverse sections, is shown in Fig. 3. It consists essentially of a broad ventral meristele and a smaller, median, dorsal meristele. These two meristeles are joined at intervals along their margins by vascular bridges. A well-developed bud trace leaves the margin of the ventral meristele close to the insertion of the bridge. This bud trace frequently gives rise to small root traces in its passage through the cortex which run into the linearly arranged rootlets behind the bud. The strands of the leaf trace, three to five in number at its origin, arise both from the bridge and from the margins of the ventral and dorsal meristeles. The space between one marginal bridge and the next constitutes the leaf gap. Root traces, other than those associated with the bud trace, arise only from the ventral surface of the ventral meristele.

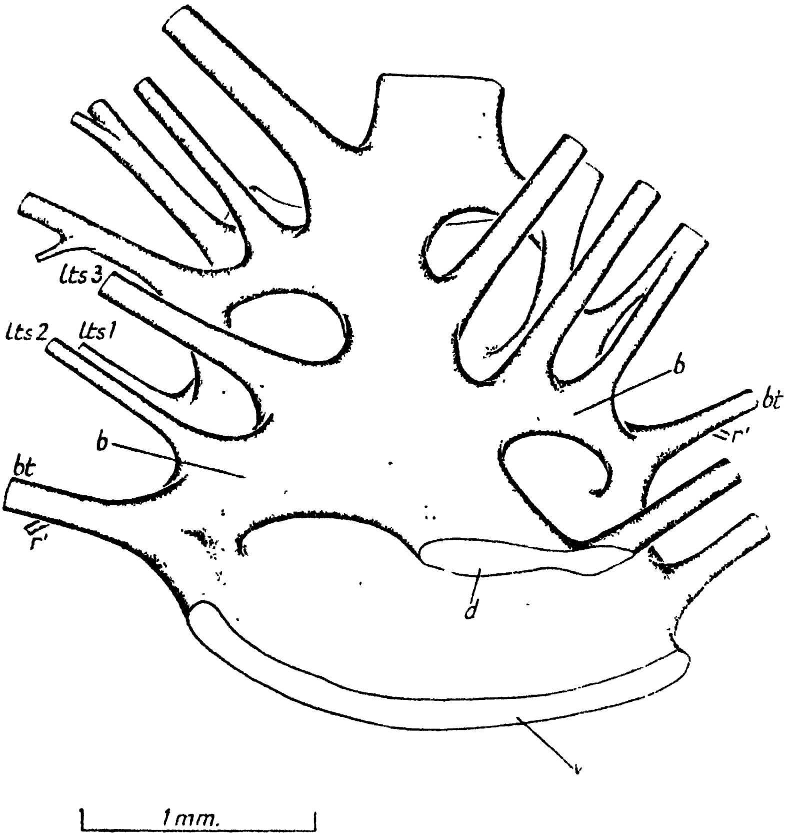

Fig. 3. *Elaphoglossum latifolium.* Reconstruction of the stele; *v*, ventral meristele; *d*, dorsal meristele; *b*, bridge connecting the ventral and dorsal meristeles; *bt*, bud trace; *lts*, strand of the leaf trace; *r'*, root in association with bud trace.

All those species examined which possess a horizontal rhizome bearing two rows of leaves have shown this type of stele. The difference between the stele of one species and that of another has been one of proportion only; there has been no change in the essential plan of construction.

In those species with an horizontal or obliquely ascending rhizome bearing more than two rows of leaves, the stele is more complex. A reconstruction of the stele of *E. muscosum* is shown in Fig. 4. The general pattern seen in *E. latifolium* is still recognizable. The ventral and dorsal meristeles, the marginal bridges, and the bud traces arising from the margins of the ventral meristele are all present. The complication arises in the region of the dorsal meristele, which appears to have been broken up by the insertion of the traces of the additional rows of leaves. The departure of these traces leaves a considerable leaf gap, but, in spite of this, the dorsal meristele more or less retains its identity. No bud traces have ever been observed to arise from the dorsal meristele, nor any buds found in association with the bases of the petioles into which the traces from the dorsal region enter.

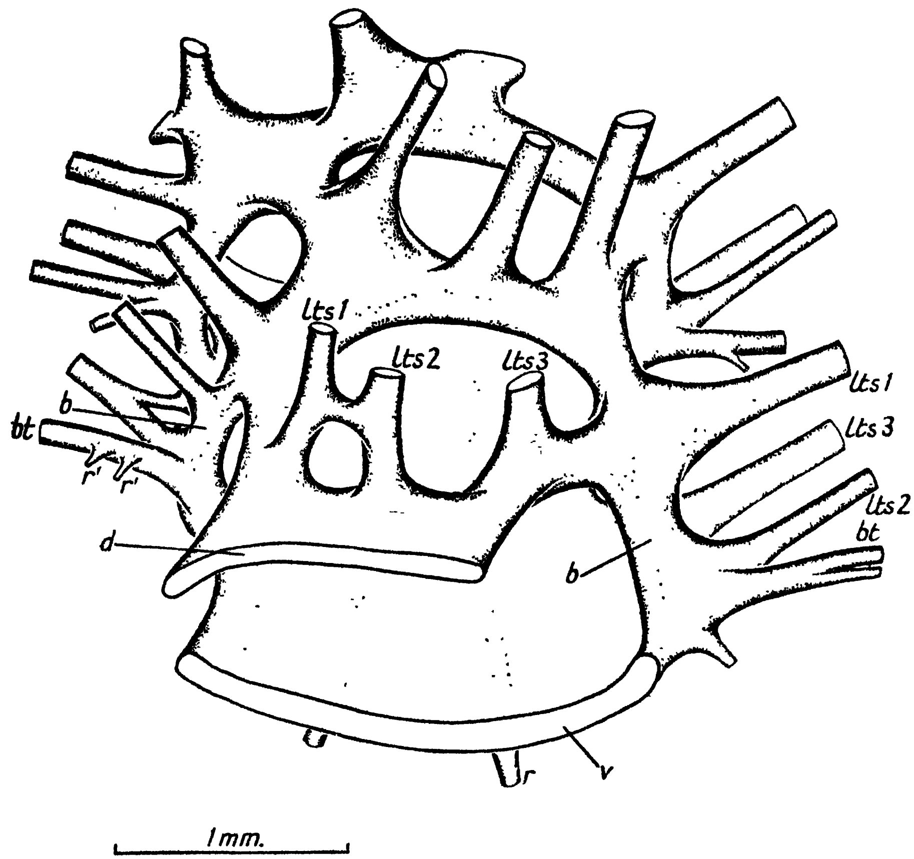

FIG. 4. *Elaphoglossum muscosum.* Reconstruction of the stele; *v*, ventral meristele; *d*, dorsal meristele; *b*, bridge connecting the ventral and dorsal meristeles; *bt*, bud trace; *lts*, strand of the leaf trace; *r*, root; *r'*, root in association with bud trace.

A stele precisely similar to that seen in *E. muscosum* was found in *E. hirtum*, but *E. pallidum* has a stele which, although closely related, is yet more complex. A transverse section of this stele is shown in Fig. 5. It is seen that the ventral meristele is still clearly present, but the stele in the region of the dorsal meristele is now very much more dissected by the insertion of leaf traces. The general pattern, however, remains recognizable and the bud traces are still found solely in association with the margin of the ventral meristele.

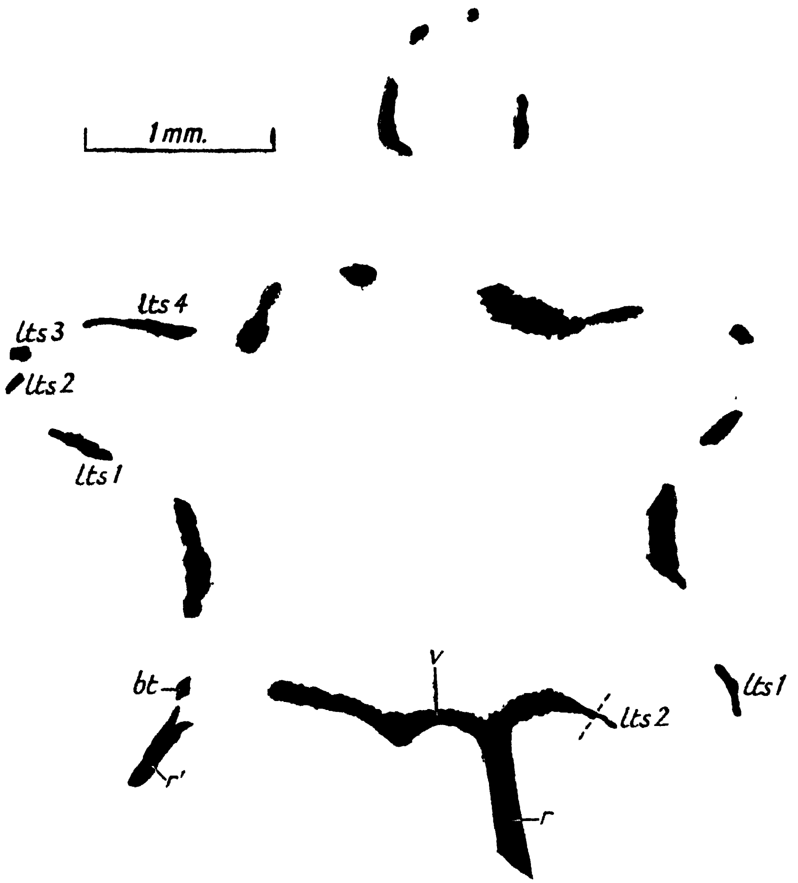

FIG. 5. *Elaphoglossum pallidum.* Transverse section of the stele; *v*, ventral meristele; *bt*, bud trace; *lts*, strand of the leaf trace; *r*, root trace; *r'*, root trace in association with bud trace.

In all these species considered so far there is a clear dorsiventrality in the stelar structure of the rhizome. The simplest type is seen in *E. latifolium.* The complex pattern seen in *E. pallidum* is attained by progressive elaboration of the dorsal part of the stele. This increasing dissection of the dorsal part is accompanied by a certain contraction of the ventral meristele and also of the degree of development of the bud trace, which is markedly less well developed in *E. pallidum* than in *E. latifolium*. There is seen in the steles of these species a tendency towards radial symmetry and dictyostely, but this tendency is far from completion.

In *E. villosum* the stelar structure is quite different. A reconstruction is shown in Fig. 6. There is no trace of dorsiventrality in the stele; it is, on the contrary, radially symmetrical and dictyostelic. Each leaf trace consists of two strands which depart from the basal margin of a relatively large leaf gap. The root traces are inserted below the sinus at the base of the leaf gap. The bud trace, where present, also departs from this region, immediately anterior to the uppermost root trace. There are no bud traces shown in Fig. 6 as it was impossible to recognize them in the series of sections from which this reconstruction was made, but, in a later series, bud traces were observed in association with three successive leaf traces (Fig. 7). The evidence upon which these traces, which are extremely tenuous and poorly differentiated, are identified as belonging to buds will be presented later in a comparative consideration of the anatomy and course of the bud trace.

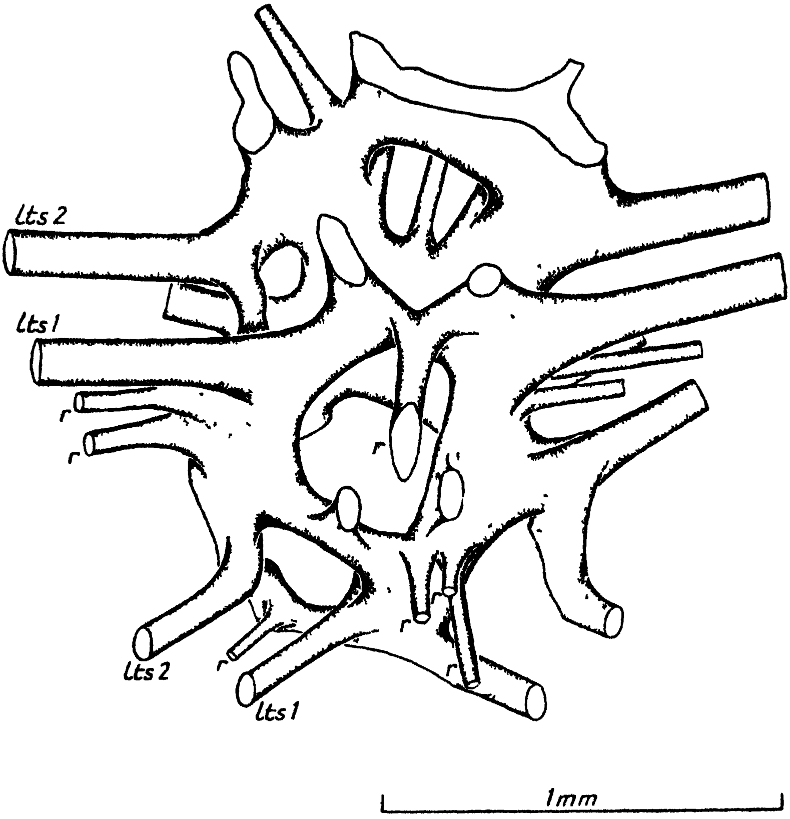

FIG. 6. *Elaphoglossum villosum*. Reconstruction of the stele, *lts*, strand of the leaf trace, *r*, root trace.

Although, as has been seen, the structure of the stele bears a relationship to the habit of the rhizome, it is evident from an examination of the apices and their subsequent development that the dorsiventral stele is not a direct modification brought about by habit. The apices of these ferns are quite striking in their flat appearance. Well-developed fronds occur only a very short distance behind the apex and fully differentiated roots may emerge almost up to point where the transverse curvature of the apex begins. Internally, although differentiation of the xylem begins very close behind the apex, there is very little spiral thickening of the tracheides; it is almost wholly annular or scalariform. In the mature tissues, too, there is only occasionally seen a protoxylem tracheide with an extended spiral or ruptured annular thickening. Both the external morphology and the internal anatomy point to the absence in the apex of any region of rapid cellular extension.

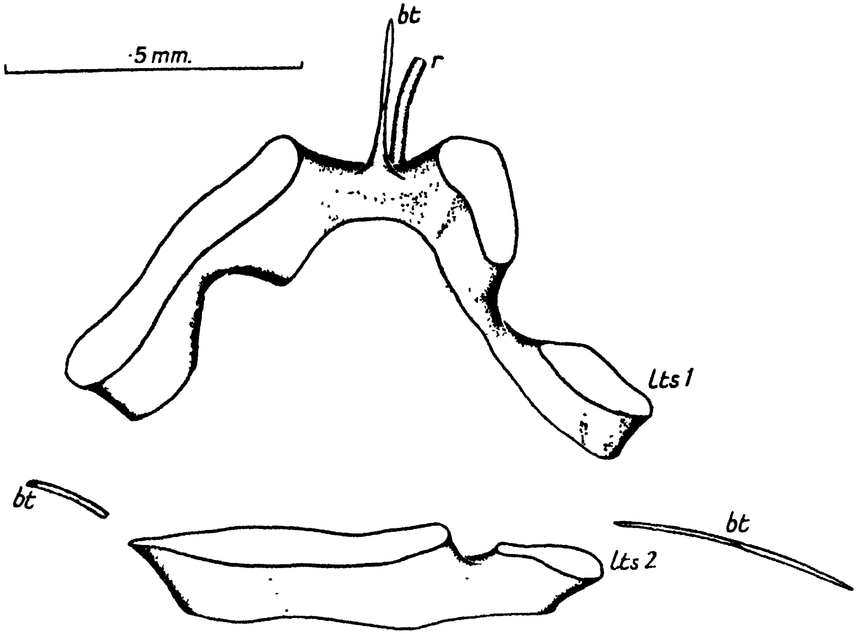

FIG. 7. *Elaphoglossum villosum*. Reconstruction of a small part of the stele to show bud traces in association with three successive leaf traces; *bt*, bud trace; *lts*, strand of the leaf trace; *r*, root trace.

Apices have been examined of all the species except *E. Sellowianum*, where no material was present. Owing to their long preservation in spirit these apices had become very hard and permeated with resin. It was not possible to embed them in wax by normal methods. Rather than experiment with so little available material it was decided to examine them by freehand sections. In every instance a single apical cell was seen at the tip of a small, but definite, apical cone. But the general symmetry of the apex was found to vary in relation to the series of stelar forms already demonstrated. A diagram of a

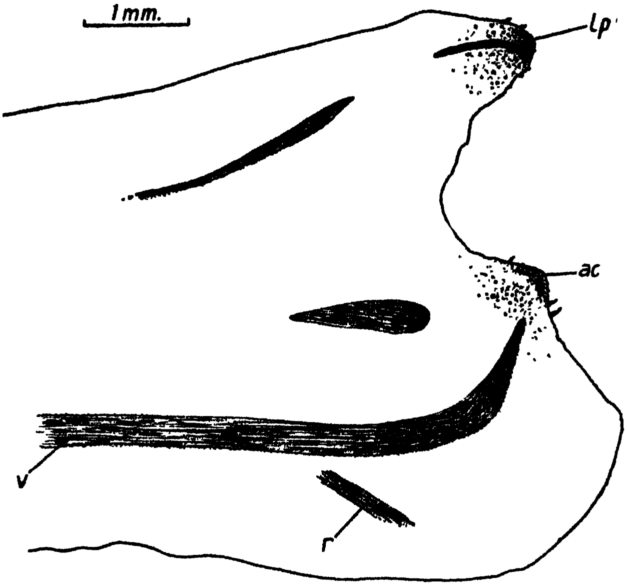

FIG. 8. *Elaphoglossum tectum.* Diagram of a median vertical longitudinal section through the apex of the rhizome; *lp*, leaf primordium; *ac*, apical cone; *v*, ventral meristele; *r*, root trace.

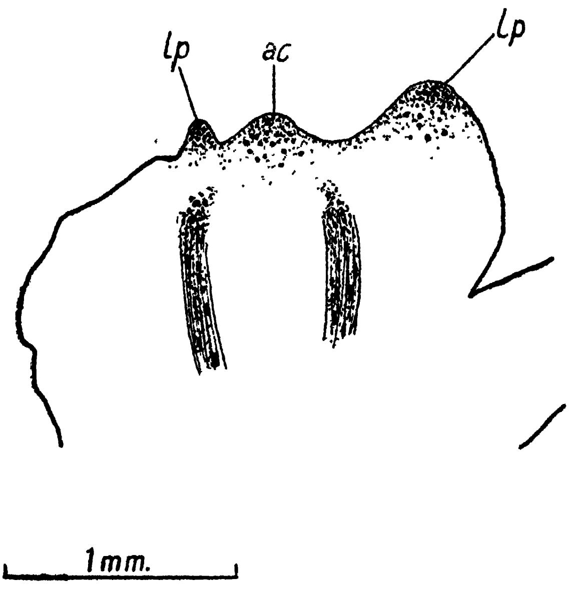

FIG. 9. *Elaphoglossum villosum.* Diagram of a median longitudinal section through the apex; *lp*, leaf primordium; *ac*, apical cone.

median vertical section of the apex of *E. tectum*, a species with a long creeping rhizome bearing two rows of leaves, is shown in Fig. 8. Here the initial tissues themselves are disposed in bilateral symmetry about the median vertical plane. The leaf primordia are confined to the dorsal side of the apical region. On the ventral side of the apex there is a marked forward protrusion of parenchymatous tissue. This protrusion possesses no area of pronounced meristematic activity and must result from a general inequality of growth about the apical meristem. The apical cone actually lies behind this ventral protrusion.

All species possessing a dorsiventral stelar structure have shown this type of bilaterally symmetrical apex. In *E. muscosum*, *E. hirtum*, and *E. pallidum*, where the rhizome bears several rows of leaves, the ventral protrusion is much less marked and may equal or only slightly exceed the apical cone.

E. villosum differs markedly from the preceding species. A diagram of a median longitudinal section of the apex is shown in Fig. 9. Here there is quite normal radial symmetry. The only structures exceeding the apical cone are the developing leaf primordia. There is nothing resembling the ventral protrusion seen in the other species.

The significance of the trends in stelar form here described and their relation to the phylogenetic trends within the genus will be considered at a later stage when all the relevant evidence is available. In the present paper the idea of progression has been used in a figurative sense and without any phylogenetic connotation.

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The Suspensor Haustoria of Some Species of *Crotalaria* Linn.

BY

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With twenty-two Figures in the Text

ABSTRACT

The structure and function of the suspensor has been studied in eight species of *Crotalaria*, in all of which it shows a differentiation into an upper and a lower part. The peripheral cells of the lower part project out and act as haustorial structures for the absorption of nutrition from the surrounding endosperm. In *C. retusa* and *C. striata* these processes are very long and tubular attacking the surrounding endosperm and nucellar tissues and extending even into the integumental layers. Remains of the suspensor are recognizable even in the mature seed. The cells of the suspensor possess chloroplasts so that this organ also has a photosynthetic function in addition to its haustorial activity.

THE genus *Crotalaria*, belonging to the tribe Genistae of the Papilionaceae, comprises nearly 350 species distributed in tropical and sub-tropical regions. Of these no less than seventy-five occur in south India alone (Gamble, 1915). Cook (1924) studied the embryology of *C. sagittalis* and Samal (1937) of *C. juncea*, well known for its fibre. Both have reported the presence of a massive suspensor in the embryo. The present investigation, which was undertaken to make a comparative study of the nature and haustorial activity of the suspensor in this genus, deals with the following eight species: *C. laburnifolia* Linn., *C. verrucosa* Linn., *C. retusa* Linn., *C. striata* DC., *C. orixensis* Rottl., *C. albida* Heyne., *C. paniculata* Willd., and *C. biflora* Linn.

The material was collected in Mysore and fixed in formalin-acetic-alcohol. The sections were stained in Heidenhain's iron-alum haematoxylin with eosin as a counterstain. In later stages it was found more advantageous to dissect out the embryo and prepare whole mounts, which gave a three-dimensional picture of this organ and showed the chloroplasts in the suspensor cells in their natural colour. The seed is cut through the micropyle into two unequal halves so as to avoid injuring any portion of the embryo. In the larger half the embryo can be seen as a green slender structure which is easily picked out with a needle. In an aqueous whole mount it is a remarkable sight to observe the suspensor wriggling about on account of the lateral protuberances of the peripheral cells stretching out after their release from the surrounding tissue where they were all along held in position. This is particularly marked in

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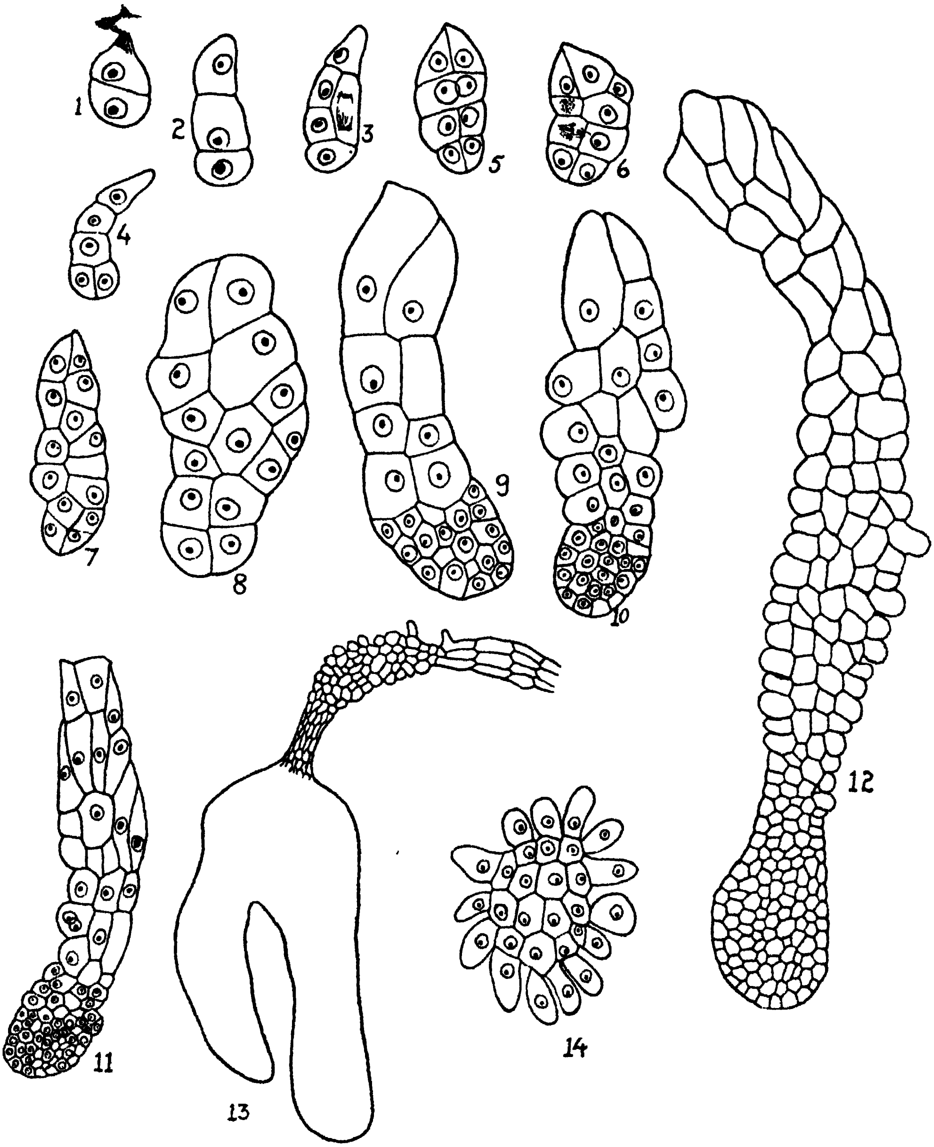

FIGS. 1–13. *C. laburnifolia*: Stages in the development of the embryo. Figs. 1–8. ×560. Figs. 9, 10. ×400. Figs. 11, 12. ×200. FIG. 13. ×80. FIG. 14. Cross-section through the suspensor. ×260.

those species in which the peripheral cells form long tubular processes. Stages older than the one shown in Fig. 11 were easily followed with the aid of whole mounts alone, some of which were made permanent by the usual methods of fixing, staining, and mounting in balsam.

OBSERVATIONS

The ovules are usually arranged in two rows on the marginal placenta. In some species the number of ovules is very small and in *C. paniculata* it is reduced to one or two. The ovules are mostly amphitropous and are sharply bent at the micropylar end. There are two integuments, of which the outer is more massive, particularly in the region of the micropyle. The inner integument is shorter and does not take part in the formation of the micropyle. It is made up of only two cell layers which also get crushed during the development of the seed so that the seed coat is formed only from the outer integument.

The fertilized egg divides transversely after a few endosperm nuclei have been formed (Fig. 1). As a rule the basal cell (the one near the micropyle) elongates and again divides by a transverse wall resulting in a three-celled proembryo (Fig. 2). The middle cell undergoes vertical and transverse divisions to give rise to a group of four cells (Fig. 3). At about the same time the terminal cell divides vertically (Fig. 4) and there is a similar division in the basal cell (Fig. 5). Hereafter, in the central region the divisions are mostly by oblique walls, but at the embryonal end they are more regular, so that an embryo-forming region (derived chiefly from the terminal cell) becomes more or less clearly demarcated from the suspensor-forming region (Figs. 6-11). The latter is itself roughly divisible into two regions, the upper consisting of greatly elongated or enlarged cells and the lower consisting of bulbous cells of which those towards the periphery project out to varying extents. This lower region of the suspensor is mostly the product of divisions of the middle cell of the three-celled proembryo. Fig. 14 shows a cross-section of the suspensor and gives an idea of its thickness. Cook (1924) reported a massive suspensor in *C. sagittalis*, but this appears to be much shorter than that of other species. In *C. juncea* (Samal, 1937) it is very long and shows the two regions clearly. The following variations in the organization and activity of the suspensor have been noticed in the species described here. In *C. albida* and *C. orixensis* the suspensor is comparatively short and the upper region shows slightly elongated cells with highly vacuolated cytoplasm. The lower region also contains enlarged cells of which the peripheral bulge out slightly and act as haustorial structures for the absorption of nutrition from the neighbouring endosperm tissue (Figs. 15, 16). In *C. verrucosa* (Fig. 17), *C. laburnifolia* (Figs. 12, 13), *C. paniculata* (Fig. 18), and *C. biflora* the suspensor is much longer. Its upper part consists of vertically elongated cells which seem to serve as haustorial structures for the absorption of nutritive materials from the nucellar tissue at the micropylar end. In the lower part the peripheral cells protrude out and absorb food from

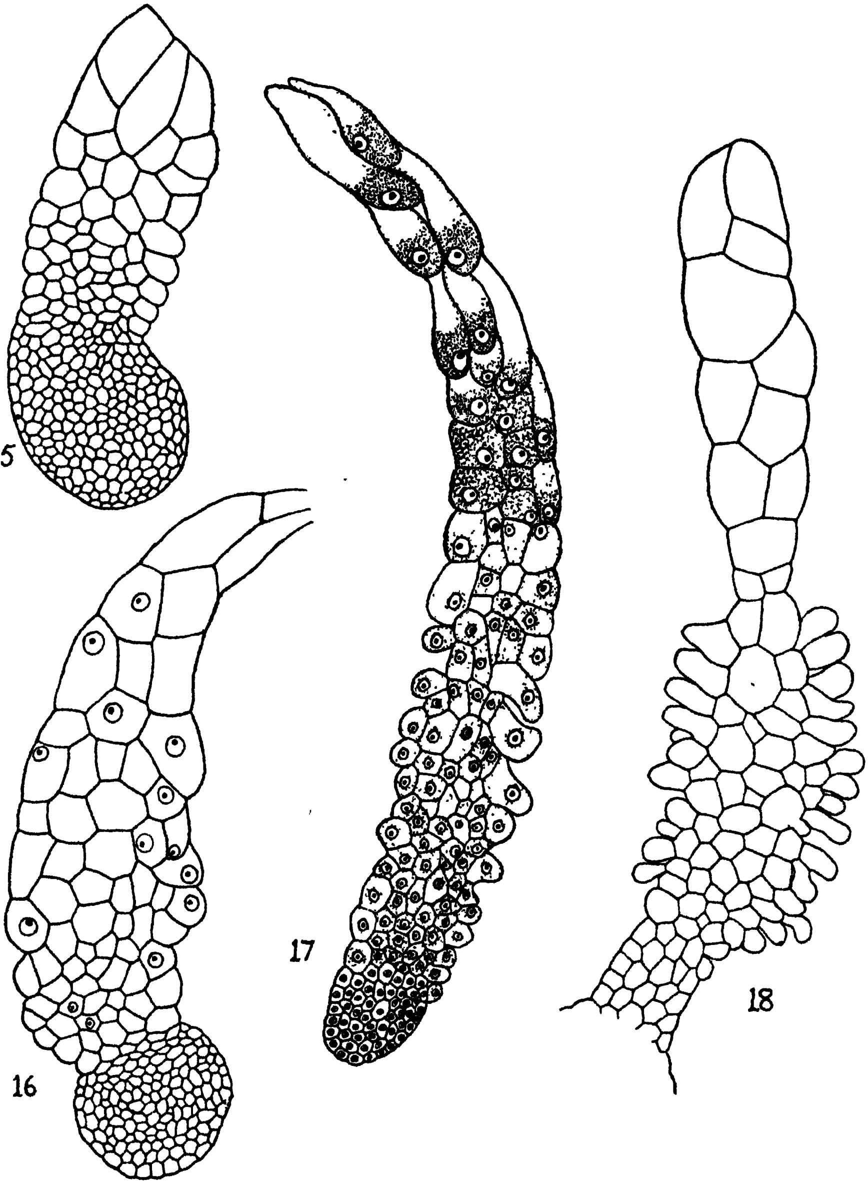

FIG. 15. *C. albida.* ×200. FIG. 16. *C. orixensis.* ×200. FIG. 17. *C. verrucosa.* ×160.
FIG. 18. *C. paniculata.* ×200.

the endosperm. Of all the species studied *C. retusa* and *C. striata* show the most pronounced haustorial activity of the suspensor. In the lower part the peripheral cells put forth tubular processes which encroach upon the endo-

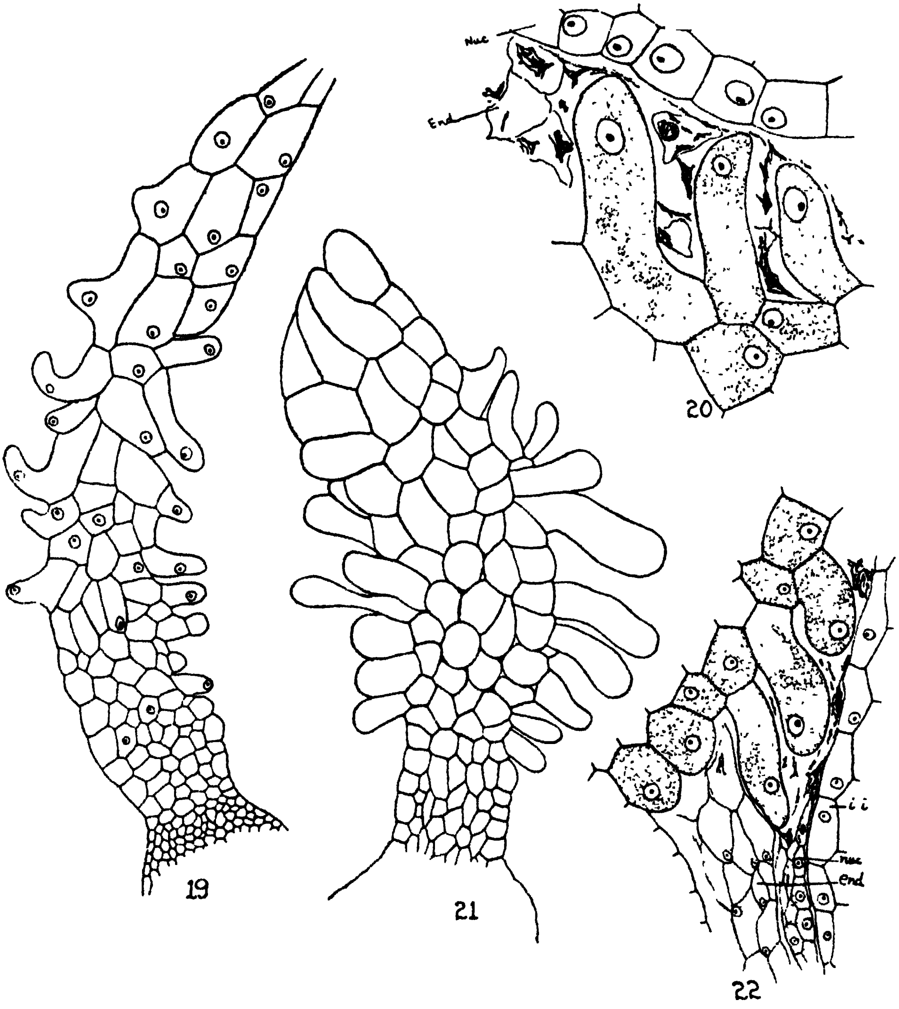

FIG. 19. L.S. of embryo of *C. retusa.* × 160. FIG. 20. More highly magnified view of the peripheral cells of the lower part of the suspensor showing their encroachment on the surrounding endosperm tissue. × 400. FIG. 21. L.S. of upper part of embryo of *C. striata.* × 200. FIG. 22. More highly magnified view of peripheral cells of the lower part of the suspensor attacking the endosperm and nucellar layers and reaching the inner layer of the integument. × 400.

sperm and then extend into the nucellar region (Figs. 20, 22). In *C. striata* (Figs. 21, 22) these tubular projections advance in every direction and reach even the integuments.

Another interesting feature, already referred to before, is the presence of numerous chloroplasts in the cells of the suspensor, so that it also serves as a photosynthetic organ in addition to its haustorial activity. The presence of chlorophyll has been previously reported by Strassburger (1880) in the suspensor cells of a species of *Lupinus*.

As already stated, divisions in the lower region of the embryo are fairly regular and result in the formation of a massive group of cells which are small and densely cytoplasmic. The cotyledons and the other embryonal regions are differentiated from this in later stages. The suspensor is recognizable even in the mature seed.

Wall formation in the endosperm takes place when the embryo has reached an advanced stage in development. The walls are first laid in the micropylar region but later extend downwards. However, even in old stages some large free nuclei can be seen at the chalazal end. Most of the endosperm is used up when the seeds are mature and the embryo occupies the entire seed cavity.

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